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they are dried. Pulverization would undoubtedly shorten the time necessary for solution.

TABLE I

Physical Characteristics of Egg White Samples

Sample	Approximate time to dissolve	Consistency	Turbidity	pH	Maximum foam volume as % original liquid	Time to reach maximum foam	Drainage in 30 minutes
	Hrs.				*%*	*Min.*	*Ml.*
Fresh white		Characteristically viscous	Clear	8.6–9.0	1100	7	6.0
Vacuum-dried without treatment	3	Thick white, retains structure and viscosity	Clear	9.6	1050	7	5.5
Chinese fermented	1½	Watery	Somewhat turbid Suspended matter	6.0	1200	5½	2.0
Acid treated, spray dried	½	Watery	Very turbid	4 6	1230	5½	0.1

The watery consistency of the commercial dried whites is a result of the treatment before drying. The structure of the thick white is completely broken down during this treatment. This reduction in viscosity appears to be one of the aims of the treatment, since it has been shown that thin white whips more readily than thick (Bailey and Le Clerc, 1935).

The turbidity of the commercial dried whites is correlated with their low pH. The pH of the acid-treated sample is identical with the iso-electric point of egg albumin. Egg white dried without treatment, on the other hand, shows a higher pH than the original fresh material, due presumably to loss of carbon dioxide during the drying. (Values below pH 8.0 were measured on a quinhydrone electrode; above pH 8.0 colorimetric methods were used.)

As might be expected, the treated samples whipped better than the fresh or vacuum-dried. Not only was the foam volume greater, but also maximum volume was obtained in less time and the foams were much more stable, as evidenced by the smaller amount of drainage. (In each case 30 g. of reconstituted white was whipped in a quart bowl with an electric mixer. The foam was transferred to measuring cups with the least possible handling, and the cups inverted in funnels draining into graduated cylinders. Whipping time was varied from

3 to 8 minutes to obtain the maximum foam volume for each kind of white.)

Meringues

To test their performance in simple meringue mixtures, three typical products were prepared from each sample, as follows:

1. Simple, uncooked frosting, prepared by beating confectioner's sugar into the white. (Thirty g. of white was whipped for 2 minutes; 50 g. of sugar was then added while beating continued over a period of 4 minutes. The volumes of the resulting stiff meringues were determined by packing in measuring cups.)

2. Boiled icing, also known as marshmallow, divinity, or heavy meringue icing, prepared by beating a hot sugar syrup slowly into whipped egg white. (Thirty g. of white was whipped 3 minutes. A hot syrup, consisting of 150 g. of sugar, 10 g. of corn syrup, and 60 ml of water, boiled to 15°C. above the boiling point of water, was poured into the whites while beating continued over a period of 5 minutes. Volume of the icing was measured as above.)

3. Baked meringues (meringue shells or kisses) for which a whipped egg white and sugar mixture is permanently set by baking in a slow oven. (To 30 g. of egg white beaten for 2 minutes, 50 g. of powdered sugar was added during beating over a period of 3 minutes. Fifteen-gram portions of the meringue were weighed onto baking sheets and baked at 275°F. for 45 minutes.)

It is to be noted that all of these products consist essentially of a whipped egg white and sugar mixture. Neither flour nor fat is used. Extensibility and heat coagulability of the protein framework are probably of minor importance even in the baked meringues, since the baking process is mainly a slow drying out.

Under these conditions the commercial treated whites were superior to the fresh or plain dried. The volumes of frosting obtained (Table II) were greater with the treated whites and the boiled icings were stiffer, maintaining peaks indefinitely. This was also true when the initial whipping period (before addition of sugar) was increased to five minutes.

TABLE II

VOLUME OF FROSTING

Egg sample	Volume of cold meringue	Volume of boiled meringue
	cc.	*cc.*
Fresh	180	300
Vacuum-dried	190	315
Chinese fermented	240	375
Acid-treated	230	380

Of the baked meringues, those from Chinese fermented white were largest, very white in color, with a compact marshmallow-like internal texture. The acid-treated were similar to the Chinese but slightly smaller in size. The fresh and vacuum-dried (practically indistinguishable) were smaller, light tan in color, and partially hollow, with a light, moist internal texture.

Batter Products

Cream-puff shells and *popovers* are particularly well adapted to a study of the baking properties of egg white, since in both products the white is essential as a source of extensible protein and the manipulation of the batter is relatively unimportant as a variable.

For both products, standard batters were prepared containing all ingredients except the egg white. The batters were then weighed out into four separate portions and one of the four types of white added to each portion. After thorough incorporation of the white, equal portions of the completed batters were weighed onto baking tins.

The commercial treated whites produced cream-puff shells less than two-thirds the size of those from fresh white (Fig. 1), and were
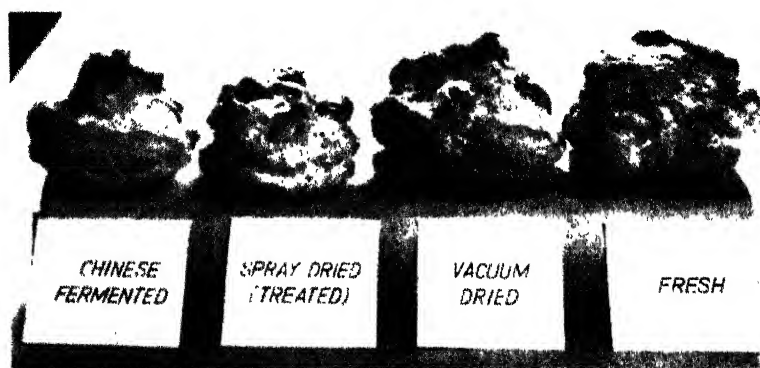

Fig. 1. Comparison of whites in cream puffs.

poorly browned and heavy walled. These shells were only slightly better than shells from which white was omitted entirely. The sample dried without treatment, on the other hand, produced shells only very slightly inferior to fresh, nicely browned shells, with thin translucent walls.

Similar but even more marked results were obtained with the popovers. Treated samples produced popovers of less than half the regular size; that is, they failed to pop. The top crust usually cracked during baking with eruption of contents and loss of steam. Popovers

from vacuum-dried whites were equal in all respects to those from the fresh material.

In *butter cakes*, the poorer baking qualities of the treated dried whites become more apparent the richer the mix, *i.e.*, the more egg, fat, and sugar for a given amount of flour. This might be expected, as the leaner formulas (approaching muffin mixes) depend mainly upon gluten development for their structural framework. Egg white may be omitted entirely with no serious effect. In the richer cakes, where the egg is essential, none of the dried whites were entirely equal to the fresh. However, the untreated vacuum-dried were a close second to the fresh in volume and texture, whereas the treated whites both produced cakes of considerably less volume with soggy streaks near the bottom.

Standard white cakes from the four types of white differed mainly in color of crust. Fresh and vacuum-dried white produced cakes with well browned crusts, whereas cakes from commercial samples were light in color as well as smaller in volume. No differences in texture were noted.

For *cakes without fat* (sponge and angel food), a whipped sugar and egg meringue that will stand up upon the addition of flour is essential, as well as proper elasticity and coagulability of the cell structure. Pyke and Johnson (1940) suggest that moderately stable meringues which drain fairly rapidly are preferable to very stable meringues with little drainage, since flour particles can be incorporated in the former in pockets between bubbles, thus contributing to the structural framework of the cake. With very stable, non-draining foams, on the other hand, the addition of the flour causes foam collapse, since only upon collapse of the bubbles is liquid made available for the flour.

The results obtained with the dried whites on angel cake support this view. The high flour and sugar formula recommended by Barmore (1936) was used, and also the method of mixing which he recommends as giving the best volume, *i.e.*, beating all the sugar with the egg white before addition of flour. The flour was folded by hand. It was noted during this folding process that the acid-treated white (most stable of the foams) was broken down more upon addition of flour than any of the other meringues. The resulting cake was smaller in volume than any of the others, with a heavy compact texture. The other three cakes were identical in volume, but the fermented white produced a somewhat harsher texture than the fresh and untreated.

Excellent whole-egg sponge cakes were obtained from fresh and untreated whites, using the single meringue method recommended by Pyke and Johnson (1940), whereas cakes containing the treated whites

were complete failures, about one-third normal volume, heavy and rubbery. This failure was due to the fact that a stable meringue could not be obtained when the treated whites were mixed with yolk. Additional evidence of the marked depressing effect of yolk on the treated whites is shown in Table III.

TABLE III

VOLUME AND STABILITY OF EGG WHITE FOAMS IN THE PRESENCE OF EGG YOLK

In each case 3 g. of fresh yolk was added to 40 g. of fresh or reconstituted white and the mixture whipped 6 minutes.

Sample	Increase on whipping	Drainage in 30 minutes
	%	
Fresh	320	3.5
Vacuum-dried	330	5.0
Chinese fermented	300 } froth on liquid	28.0
Acid-treated	180 } layer	32.0

The serious effect of small amounts of yolk in destroying foaming of the dried whites has been traced to the low pH of the latter. When the pH of the acid-treated white was adjusted to 9.0 by addition of sodium hydroxide to the water used for reconstituting, a volume increase of 470% was obtained on whipping with yolk and no drainage was obtained. Barmore (1936) mentions the fact that egg yolk reduces the foaming of fresh egg in acid solutions much more than at the original pH of the white.

However, the generally poorer baking qualities of the treated samples of dried white cannot be ascribed entirely to their low pH. The only product which was greatly improved by the use of the alkalized white was the whole-egg sponge cake, which was equal in volume and texture to the fresh and vacuum dried. Cream puff batter was thicker with alkalized than with the regular treated white, resulting in a more knobby appearance to the baked puff, but the volume was not improved. Popovers showed little or no improvement. Whipping volume was greatly decreased (800% as compared to 1230%) so that the alkalized material would be unsuitable for those products (meringues, confections, etc.) where the treated whites were found to be advantageous.

Probably a lack of heat coagulable protein, owing to partial hydrolysis of the acid-treated and fermented materials, is the chief cause of their failure in baking. Increasing the amount of dried white used in cream puffs from 13% to 20% produced a puff comparable in volume to that from fresh white, provided the pH of the white was adjusted to 9.0. Increasing the concentration of dried material without adjust-

ing the pH caused extreme thinning of the batter, so that the resulting puffs were smooth and flat, without the characteristic knobbiness of shells from fresh white.

Discussion

This work demonstrates that dried white can be used almost, if not quite, as successfully as fresh white for general baking purposes, provided white from good-quality eggs is dried quickly at a temperature below the coagulation point, and without previous treatment that has caused denaturation of the protein.

From a practical standpoint it would be desirable to have a simple test by which the baking qualities of commercial samples of dried white could be rated. Lacking suitable physical or chemical tests, it is suggested that a popover baking test would probably give the needed information most quickly and certainly, and with little expenditure of materials.

Sufficient batter, including all ingredients except the white, should be prepared for all the samples of white to be tested. The formula adopted in this laboratory is as follows:

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All purpose flour................... 100 g.
Water............................... 200 g.
Egg yolk............................  34 g.
Salt................................   2 g.
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To each 100 g. of batter (mixed until smooth) is added 30 g. of one of the whites to be tested. (A control is conveniently run on another 100-g. portion of the same batter, using 30 g. of fresh white.) After thorough mixing of the white, 20-g. portions of the completed batter are weighed into muffin tins and baked for 20 minutes at 450°C., and another 20 minutes at 350°C.

A number of commercial dried whites have been tested in this way. Some of these had obviously been fermented. The history of other samples was not known. No material known to be enzyme treated could be obtained. All of the commercial samples tried produced very poor popovers, similar to those made from the acid-treated and Chinese fermented and far inferior to the samples dried in this laboratory.

Obviously, partially hydrolyzed, dried material with high whipping ability has an accepted place in the preparation of various types of meringue mixtures. A simple whipping test for foam volume and drainage is widely employed for testing dried whites to be used for this purpose, according to Le Clerc and Bailey (1940). This test is of course useless in determining the suitability of the white for baking. It is suggested that whites suitable for baking should be dried as a

separate article and carefully tested to see that they retain their baking characteristics.

Summary

Commercial samples of dried white were found to whip better and to be more suitable for meringue mixtures than fresh white or vacuum-dried material prepared in the laboratory. The increased whipping ability of the commercial whites is believed to be due to the partial hydrolysis brought about by special treatment (fermentation or acid treatment) given them before drying.

On the other hand, in all batter and dough products the commercial treated whites were greatly inferior to fresh or laboratory-dried whites. This inferiority was traced to their low pH and decreased amount of heat-coagulable protein.

Laboratory-dried (untreated) white was found to be only slightly inferior to fresh white for baking. A simple baking test to determine the suitability of dried whites for batter and dough products is proposed.

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STANDARDIZATION OF THE EVELYN PHOTOELECTRIC COLORIMETER FOR FLOUR PIGMENT DETERMINATIONS [1]

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(Read at the Annual Meeting, May 1940)

Determination of the pigment content of flour extracts presents a special type of problem due to the fact that two groups of pigments are present, differing rather widely in their spectral properties. Until recently, it has been generally considered that carotene constituted the principal yellow pigment of flour; the work of Markley and Bailey (1935 and 1935a), however, indicates that wheat and flour pigments are a highly complex mixture, containing alpha and beta carotenes, xanthophyll and its esters, tricin and similar flavone pigments, together with other unidentified yellow coloring matters of the flavone type. An examination of the spectral distribution curves for typical high-grade-flour extracts, however, suggests that the pigments present are substantially carotenoid in nature, and recent unpublished studies by the senior author indicate that the major carotenoid pigment is xanthophyll or its esters, rather than carotene itself. The flavone pigments appear to be closely associated with the bran, and therefore appear in increasing amounts in lower-grade flours.

The problem of estimating the pigment content of a flour extract is thus not a simple one and cannot be adequately solved by simple colorimetric means. Of the various methods proposed, the spectrophotometric procedure of Ferrari and Bailey (1929) most closely approximates the ideal, since the estimation of carotenoid pigment is based upon a measurement of the transmittancy of the extract for monochromatic light of a single wave length (4,358 A. U.) at which point the absorption is very high. Under such circumstances, the interfering effect of flavone pigments is reduced to a minimum, and the method, while not specific for either carotene or xanthophyll, gives an excellent indication of the *total* carotenoid pigment present. Unfortunately, the equipment required is expensive, and the method in general is better adapted to research investigations than to routine determinations. It represents, however, the fundamental standard procedure to which all other methods should be compared.

The simplified colorimetric procedure described by Geddes, Binnington, and Whiteside (1934) does not require elaborate apparatus, especially in view of the production in recent years of inexpensive

mercury vapor lamps.   No standardization other than the preparation of known potassium chromate solutions is required.   It is, however, a visual procedure, and as such is subject to the limitations and inaccuracies inherent in all methods employing the eye as a detector of photometric balance.

In recent years various types of so-called photoelectric colorimeters have appeared upon the market, and a number of workers in the field of flour pigments have turned to these instruments as a means of increasing the precision and rapidity of their tests.   Most such devices are designed primarily for use with standard colorimetric procedures where a white light source and filters transmitting relatively broad regions of the spectrum are adequate; such apparatus is not well adapted to flour pigment determinations, since the sensitivity of the instrument is not great enough to provide adequate galvanometer response when a sharp-cutting filter of low total light transmission is employed.   The use of such filters is essential if the interfering effects of flavone pigments are to be minimized, and if they are not employed separate calibration curves must be prepared for each grade of flour examined.

The most serious objection to the use of photoelectric instruments resides in this question of standardization.   In conventional colorimetric procedures, calibration curves can be readily prepared by developing a series of color standards with graded known amounts of a reference material, but such methods are not applicable to flour carotenoids.   Some workers have employed beta-carotene as a standard, but this practice is open to serious question, since the composition and spectral characteristics of the standard and the unknown differ widely.   Furthermore, the reliability of commercial beta-carotene as a standard is very doubtful unless it is carefully purified.   Under such circumstances, the only reliable standards would appear to be a series of flour extracts whose pigment content had been previously determined by means of a spectrophotometer.   As equipment for such a proceeding is not generally available, the utility of photoelectric methods would appear to be very limited, unless instruments could be obtained in which the agreement between duplicate models was sufficiently good to permit of constructing a permanent calibration curve.   It is believed that the Evelyn (1936) Photoelectric Colorimeter [2] meets these requirements.

Since the Evelyn instrument was originally developed for biological studies and is not as well known to most workers as some other types, a brief description of the apparatus and its advantages for this particular type of problem is here presented.   It is a direct-reading in-

[2] Manufactured by the Rubicon Co., 29 North Sixth St., Philadelphia, Pa.

strument employing an exceedingly sensitive and well-damped gal-vanometer in conjunction with a 6-volt lamp and a barrier-layer type of photocell, thus enabling the use of very low light intensities. Several advantages are manifest in such a system; the low intensity permits of working in the linear portion of the photocell-output curve, thus minimizing fatigue and hysteresis effects. Secondly, it enables the use of comparatively sharp-cutting filters, isolating fairly narrow spectral regions. Thirdly, the current consumption of the lamp is so small as to permit of using a storage battery for long periods of time without recharging and thus provides a very stable illuminating system free from the effects of line voltage variations or drift due to heavy battery drain. The use of filters is an integral part of the instrument, and it cannot be employed with white light alone; furthermore, the absorption cells are merely specially selected $7 \times \frac{7}{8}$-inch test tubes, obviating the necessity of using fragile and expensive cells of special construction.

With the above points in view, the present study involving calibration with both naphtha+ethyl alcohol (93 : 7) and water-saturated butanol solvents was undertaken.

Experimental

To check the variation between duplicate instruments, a comparison was made between stock models available in this laboratory and

TABLE 1

COMPARISON OF THE "APPARENT DENSITIES" (L) OF POTASSIUM CHROMATE SOLUTIONS OBTAINED WITH TWO EVELYN PHOTOELECTRIC COLORIMETERS

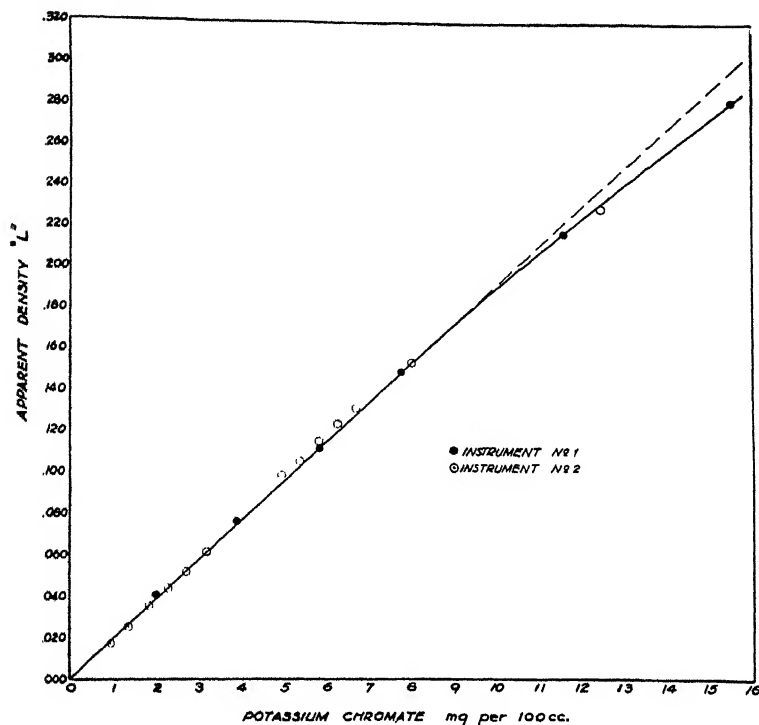| K₂CrO₄—mg. per 100 cc.	Apparent density (L)	
	Instrument No. 1	Instrument No. 2
0.92		.0166
1.36		.0246
1.81		.0351
1.94	.0398	
2.26		.0434
2.70		.0518
3.14		.0617
3.88	.0757	
4.91		.0982
5.36		.1051
5.80		.1149
5.82	.1177	
6.24		.1235
6.68		.1308
7.75	.1487	
8.00		.1534
11.64	.2164	
12.50		.2291
15.52	.2819	

Fig. 1.   Comparison of two Evelyn colorimeters.

in the research laboratory of the Ogilvie Flour Mills Co., Montreal, Canada.   Both instruments were equipped with compound glass filters having a peak transmission of 440 millimicrons and a total transmission range of approximately 60 millimicrons.   Because of the possibility of change in the transmittancy of flour extracts during shipment, the comparisons were made with dilute potassium chromate solutions prepared on the spot by each collaborator using the reagent-grade chemical, distilled water, and laboratory-grade glassware.   Results of these comparisons are presented in Table I and graphically in Figure 1. It will be noted that the concentrations selected by the two operators were not identical and the best comparison is therefore shown in the graph.   The tabulated data, however, should be of value in checking the uniformity of other instruments of this type.   These results suggest that a high degree of uniformity exists between various instruments of this manufacture and therefore that a carefully worked out standardization for flour pigments should be of general utility.

General Technique of Standardization

The extracts employed in making the comparisons were freshly prepared, using both naphtha+absolute ethyl alcohol (93 parts to 7 parts) and water-saturated *n*-butyl alcohol. In the instance of naphtha+alcohol, the procedure described in *Cereal Laboratory Methods* (1935) was followed, involving a 20-g. sample of flour, 100 ml. of solvent, overnight extraction, and centrifuging. The flours employed were all commercially milled and included some first and second clears.

The butyl alcohol extracts were prepared with commercial butanol according to the procedure described by Binnington and Geddes (1939). The same sample-to-solvent ratio as with naphtha+alcohol was employed, with a 15-to-30-minute extraction time. Clarification was effected by filtration through No. 1 Whatman paper. The flours used in this group of tests were similar to those employed in the naphtha+alcohol study, with the addition of a number of experimentally milled unbleached samples supplied by the Division of Agricultural Bio-chemistry, University of Minnesota.

Pigment content of the extracts was determined with a König-Martens spectrophotometer, the transmittancies obtained being calculated into terms of carotene, using the values 1.91565 and 1.6632 for the specific transmissive indices of carotene in naphtha+alcohol and water-saturated *n*-butyl alcohol respectively. All readings were made at a wave length of 4,358 A. U. The spectrophotometric results are thus expressed in terms of carotene. It has been previously indicated that the pigments of wheat and flour are complex and expression of the transmittancy in terms of carotene can therefore only be considered as an approximation. Within the grades of flour examined, however, the spectral properties are essentially carotenoid, and the specific transmissive indices of carotene and xanthophyll are so close together that even if *all* the pigment were xanthophyll the error would only be in the order of 6%.

Readings with the Evelyn instrument were obtained by the following procedure: The illumination was turned on 30 minutes prior to making determinations to insure constancy of readings. A tube of the appropriate solvent was then introduced and the galvanometer deflection adjusted to a value of 100 on the scale. The solvent tube was then removed, the sample substituted, and the galvanometer reading noted. Such readings are numerically equivalent to percentage transmittancy, and when plotted yield a logarithmic curve. Accordingly they were converted to apparent densities by reference to a table supplied with the instrument. Density equals the negative logarithm of the transmittancy and a plot of such values is therefore linear over the region in which the solution obeys Beer's law.

TABLE II

Correlation coefficient between spectro-photometric carotene (ppm. in flour) and apparent density (L) as determined with the Evelyn photoelectric colorimeter	$r = +.997$ (1% point $= 0.275$ approx.)
Regression equation: Carotene — ppm. (naphtha + alcohol solvent) $= 0.046 + 0.1478$ L (apparent density)	Standard error of estimate 0.048 ppm.

NAPHTHA + ALCOHOL (93:7)

APPARENT DENSITY "L"

CAROTENOID   PIGMENT   P.P.M. IN FLOUR
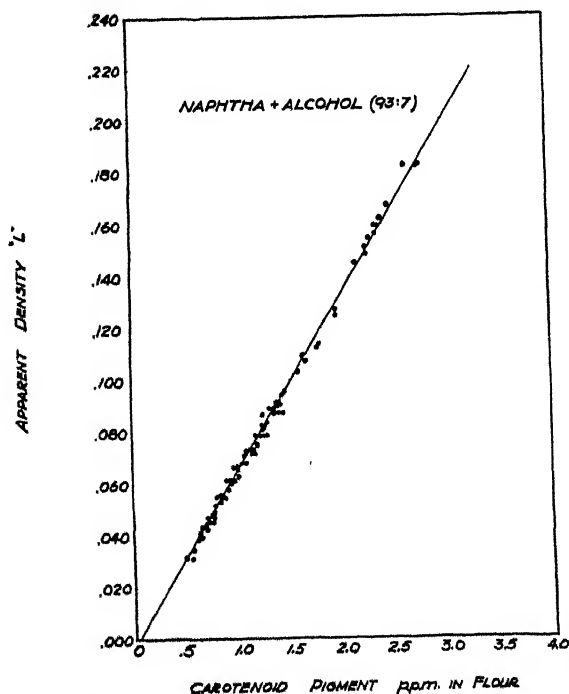
Fig. 2.   Statistical data from 87 flours—naphtha+alcohol.

The results obtained were submitted to statistical analysis, and coefficients of correlation, regressions, and standard errors of estimate were computed.   The results obtained with naphtha+alcohol are presented in Table II and the individual data together with the computed regression line are plotted in Figure 2.   This regression line represents the calibration curve and may be used as such.   It is preferable, however, to construct a table by listing the values of G (galvanometer

reading) in 0.25-unit increments, listing the corresponding values of I, and computing the carotenoid pigment equivalents from the regression equation. Inspection of the plotted data indicates that close correspondence exists between spectrophotometric and photoelectric results, and the low standard error of estimate (0.048 ppm.) indicates that estimation of flour carotenoids may be made with high accuracy.

The results obtained with water-saturated butanol as a solvent are presented in Table III and the graphical relations illustrated in Figure 3. A greater deviation of the points from the regression line will be noted here than in the instance of naphtha+alcohol. This increased deviation is reflected in a larger error of estimate. However, the amount of pigment extracted by butanol is appreciably greater than in the instance of naphtha+alcohol, and this tends to reduce the difference when expressed on a percentage basis. The inclusion of additional data secured with a number of experimentally milled samples also has a tendency to cause a slight increase in the error of prediction. Although this error is approximately twice as great as the comparable value for naphtha+alcohol, both are so small as to be insignificant from a practical standpoint. Additional values are plotted in Figure 3, representing results obtained with 17 samples of ground durum semolina which were not included in the regression. These values fall very close to the regression line, indicating that this calibration may be employed in the analysis of durum semolinas.

Discussion

From the evidence presented, the Evelyn photoelectric colorimeter appears to be particularly well suited to the estimation of carotenoid pigments in flours. It is stable and extremely sensitive and may be equipped with suitable filters. In addition the two instruments of this manufacture examined are in sufficiently close agreement to justify the use of a calibration curve established with a single instrument. This last is a very important point, since one of the major objections to photoelectric colorimeters for this class of work is the difficulty of standardization, which ordinarily can only be satisfactorily effected if a spectrophotometer is available. However, the pigment calibration made in this study may be applied to other instruments of the same manufacture if it has previously been found that calibration of the instrument in question against potassium chromate standards agrees with the comparable data presented in this paper.

In the standardization of this instrument with extracts prepared with naphtha+alcohol and butyl alcohol respectively, the results obtained with the latter solvent show a wider scatter than the former. As the photoelectric results are secured by measurements obtained with

TABLE III

Statistical Data for Standardization of the Evelyn Photoelectric Color-
imeter for Carotenoid Pigments in Flour Extracts Obtained with Water-
Saturated *n*-Butyl Alcohol in the Instance of 103 Flours

Correlation coefficient between spectro-photometric carotene (ppm. in flour) and apparent density (L) as determined with the Evelyn photoelectric colorimeter	$r = +.997$ (1% point = 0.254 approx.)

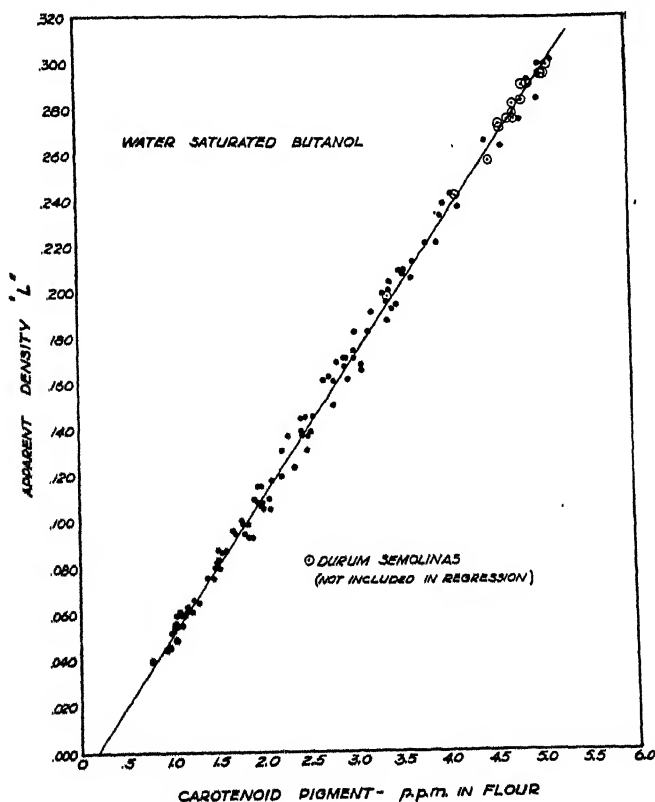| Regression equation: Carotene ppm. (water-saturated butanol solvent) = 0.174 + 0.1657 L (apparent density)	Standard error of estimate 0.087 ppm.

Fig. 3.   Statistical data from 103 flours—water-saturated butanol.

a broader spectral region than is the case with the spectrophotometer, it is to be inferred that the butyl-alcohol solvent extracts rather more flavone pigments than the naphtha+alcohol. This conclusion is in opposition to that of Binnington, Sibbitt, and Geddes (1938), who believed that butyl alcohol was more satisfactory in this respect.

Summary

The complex nature of the pigments of wheat and flour is pointed out and certain factors involved in their estimation in flour extracts are considered. The inapplicability of conventional colorimetric procedures to this type of determination is indicated.

Various methods of estimating the pigment content of flour extracts are discussed and the spectrophotometric procedure indicated as most closely approximating the ideal. It is pointed out, however, that it represents a research procedure and that it is not well adapted to routine testing.

The use of photoelectric colorimeters is discussed, their limitations indicated, and the difficulty of standardization pointed out. A particular type of photoelectric instrument, the Evelyn, is briefly described and the agreement secured between two instruments of this manufacture is detailed. This agreement is sufficiently good to permit the use of a single calibration curve with the instruments employed. In the event that these calibration data are to be applied to other instruments of the same manufacture, a check with potassium chromate solutions is suggested.

The results of an extensive series of comparisons between spectrophotometric and photoelectric results are detailed and data presented enabling the construction of calibration tables and curves for both naphtha+alcohol and water-saturated n-butyl alcohol solvents. The errors of prediction are 0.048 and 0.087 ppm. respectively for the two solvents. It is pointed out that from a practical standpoint, these errors are of an essentially similar order of magnitude.

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THE ORIGIN OF THE GAS CELL IN BREAD DOUGH

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(Read at the Annual Meeting, May 1940)

This paper is a study of the conditions and causes that produce gas cells in dough and control their retention.   A preliminary survey of bread making would indicate that the cells in dough originate for the following reasons:

1. The gas cells in the endosperm particles are incorporated in the dough.

2. The gas voids between the endosperm particles in the flour are occluded in the dough during mixing.

3. The mixing beats gas into the dough and subdivides it to produce gas cells of small size.

4. The gas pressure caused by yeast will originate new gas cells around the organism.

5. The work applied to the dough after it is mixed, such as folding, punching, rolling, molding and twisting, subdivides gas particles to increase their number.

To test the above hypotheses and to indicate their relative importance, means were designed whereby bread could be produced in which all of the operations of bread making were eliminated as far as possible, thus enabling one variable to be studied at a time.   For this purpose "no-time" doughs were employed for the first part of this paper.   The molding of doughs or other means which apply work to the dough beyond the mixing operation were eliminated.   The dough, immediately after mixing, was gently extruded through a 1⅜″ orifice directly into the pan.   This manner of shaping the loaf involves no substantially additional work beyond the mixing process.   The loaves were then proofed to standard height and baked.

In order that satisfactory no-time breads can be made, rapid-acting oxidizing agents are necessary, which are capable of producing satisfactory bread with ordinary mixing procedures. For this purpose one can use vanadate catalyst with bromate, as described by Mac-Intosh (1937), or sodium chlorite can be used according to the process of Hans Pelser (1930). In these experiments sodium chlorite was used. The bread obtained by this no-time process is nearly identical to that obtained by the use of bromate in a two- or three-hour straight-dough process. The main difference is in flavor.

To test the above hypotheses a dough of standard no-time composition was mixed in air with the optimum amount of sodium chlorite and mixing time to produce the best loaf possible from the flour. The same standard baker's patent was used throughout this series of experiments. A similar dough was mixed under identical conditions

Mixed in air   Mixed in vacuum
Figure 1

except that the mixing was conducted in vacuum, as described by Baker and Mize (1937). The two no-time loaves are shown in Figure 1.

The first loaf, the air-mixed loaf, is normal bread. A similar dough when mixed in vacuum and baked [1] gives the second loaf, which is coarse-textured and thick-walled. The cell walls are entirely devoid of small gas cells, there being no other gas cells in the bread than are visible to the eye in the picture. The walls appear very similar to the beeswax in honeycomb. It is obvious in this bread that either very few cells were present at the end of the vacuum mixing or few were produced by the yeast itself. It is also obvious that the yeast under these conditions is incapable of creating gas cells in a sufficient number to produce ordinary bread. It is very apparent that once a few gas cells are available, the gas produced by the yeast organism diffuses into them and such cells receive the gas of fermentation without creation of further cells. The dough had an expansion during the proofing period equal to that of a dough produced by the ordinary

[1] Baker and Mize (1937) obtained good bread from such vacuum mixes by using 2½-hour straight-dough fermentation methods.

mixing method. One might conclude from this experiment that the yeast organism is incapable of originating gas cells in dough.

The density of the vacuum-mixed dough coming from the mixer was 1.25, and that of the air-mixed dough was 1.15. The difference between these two densities of 0.10 would indicate that the air-mixed dough had about 8% of its volume occupied by gas voids or cells which were produced in the mixing, contrasted to the vacuum dough which obviously will contain no such gas spaces. Possibly the texture obtained in the air-mixed dough occurred because sufficient corresponding small gas voids or cells were produced during the mixing.

Figure 2 shows exactly similar bread with the exception that the first two loaves were mixed in carbon dioxide, of which the first, immediately after extruding into the pan, was placed in a chamber and subjected to air pressure of 80 pounds per square inch for five minutes.

Mixed in CO_2, then subjected to   Mixed in CO_2 Mixed in vacuum
an air pressure of 80 lbs./sq. in.

Figure 2
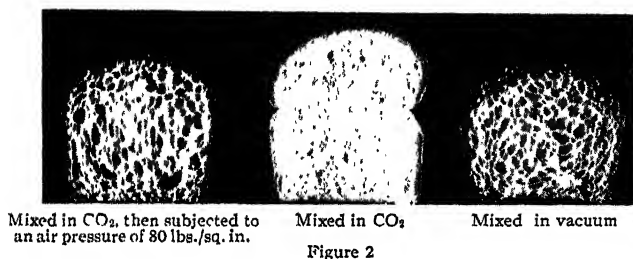
The third loaf was mixed in vacuum. The three loaves were proofed to standard height and baked.

It is to be noted that the vacuum loaf is similar to the one described above. The carbon dioxide loaf is similar to the air-mixed loaf, with possibly slightly improved texture. The first loaf, which was also mixed in carbon dioxide and then subjected to pressure, appears identical with the loaf mixed in vacuum. It is to be noted that the density of the loaf coming from the pressure chamber was 1.22, whereas going into the chamber it had a density of 1.15. Obviously the pressure has driven the gas contained in the gas cells or occluded in the dough into solution and has produced a solid compact dough similar to that mixed in vacuum.

The only difference observable between the carbon dioxide pressure loaf and the vacuum loaf in this instance was that the pressure loaf proofed in a slightly shorter time, doubtless because of its larger initial supply of carbon dioxide. It is again obvious that the yeast organism is unable to originate gas cells to produce bread texture. It is also obvious that the texture present in the second loaf must have been present in the dough coming out of the mixer. The 8% of gas volume

present in this dough is apparently distributed as extremely small voids or cells which can be considered as gas nuclei, which, upon expanding, as gas feeds into the cells, produce the resulting texture in the bread.

Figure 3 shows the same two loaves of bread mixed in carbon dioxide as Figure 2, subjected to the same treatment of no pressure and pressure. Loaves 6 and 7 of this chart are two exactly similar loaves mixed in air. The pressure which was applied to Loaf 7 was exactly the same as that applied to Loaf 5, but when released gave a dough of a density of 1.17. Very little effect was produced upon the texture of the resulting bread, only coarsening or damaging it to a slight

Mixed in CO₂

Mixed in air

No pressure   Subjected to an air pressure
                   of 80 lbs./sq. in. after panning
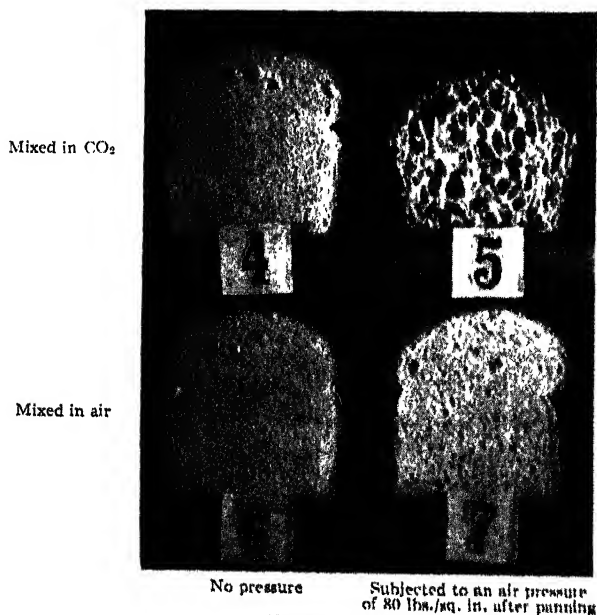
Figure 3

degree. It is obvious that air, being a relatively insoluble gas, was not forced into solution by the pressure and the voids or cells in the freshly mixed dough were thus not eliminated. Hence the dough retained the gas cell nuclei which were produced in the mixing process. Further illustration and proof are shown in the following figures.

Figure 4 shows a series of doughs mixed in carbon dioxide which were subjected to increasing pressures up to 80 pounds per square inch for five minutes, showing clearly that as the pressure increased and approached 80 pounds the number of gas nuclei present rapidly decreased and approached the vanishing point.

No pressure   5 lbs./sq. in.   10 lbs./sq. in.

20 lbs./sq. in.   40 lbs./sq. in.   80 lbs./sq. in.
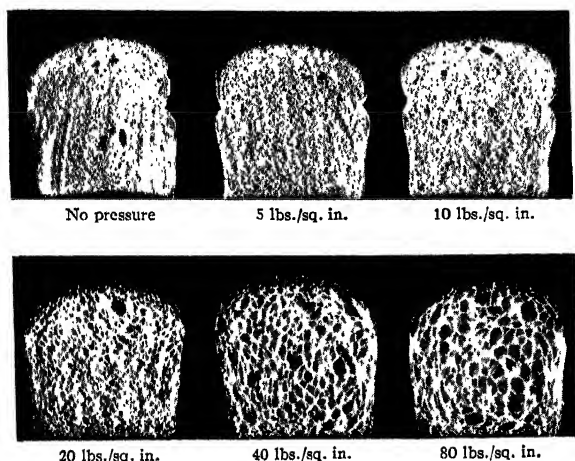
Fig. 4.   Effect of air pressure applied immediately after panning, to doughs mixed in carbon dioxide.

Before proof   One-half proof

Full proof   No pressure
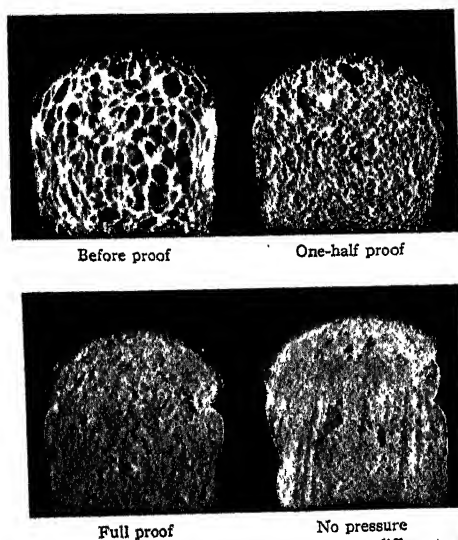
Fig. 5.   Effect of air pressure applied at 80 lbs./sq. in. at different periods during pan proof on doughs mixed in carbon dioxide.

Figure 5 illustrates the same point in a different manner.   A series of carbon-dioxide-mixed doughs were extruded and then subjected to pressure at different times during proofing, from no proof to full proof, 80 pounds pressure being applied for five minutes in each case.   As the volume of carbon dioxide in the dough increased with fermentation the pressure was unable to drive it all into solution and many of the

gas nuclei, though doubtless reduced in individual size during the compression, contained too much gas to be entirely dissolved and thus retained their identity.

Figure 6 shows a loaf mixed in vacuum and three other loaves which were mixed part of the time in vacuum and part of the time in air. Loaf 1 was mixed seven minutes in vacuum, Loaf 2 four minutes in vacuum and three minutes in air, Loaf 3 six minutes in vacuum and one minute in air. Loaf 4 was exactly similar to Loaf 3 except that the yeast was added only during the last minute of mixing. It is obvious that the gas cells and nuclei which were eliminated in the
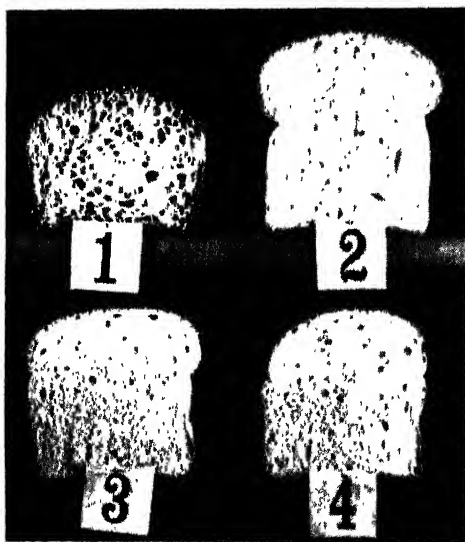
Fig. 6. Functions of the mixing period: (1) vacuum mix 7 min., (2) vacuum mix 4 min., then air mix 3 min., (3) vacuum mix 6 min., then air mix 1 min., (4) vacuum mix 6 min. without yeast then air mix 1 min. with yeast.

vacuum mixing were returned to the dough almost completely in one minute of mixing in air and were completely returned to the dough in three minutes of mixing in air, producing a loaf equal to or better than the seven-minute optimum for this dough shown in other figures. It is also apparent that the presence or absence of yeast has little effect upon this mixing. It is obvious that one can destroy all gas cells and nuclei in dough which may have originated from gas voids in the endosperm or which may be occluded from gas carried between the flour particles or which were beaten in during the early stages of mixing, and entirely replace or substitute for such gas voids, those produced during a relatively short period of mixing in air.

It now becomes apparent that the purpose of mixing doughs is not primarily that of emulsifying gas. The full seven minutes of mixing time which was required to produce good bread from these doughs is apparently not needed for emulsification purposes. Evidently the mixing must do something else to the dough.

Figure 7 was a study designed to show the effect of mixing time upon oxidized and unoxidized doughs. The left half of the figure with the lower numbers are all unoxidized doughs. The right half, with the higher numbers, are all doughs oxidized to produce an optimum loaf when mixed in air for seven minutes. All doughs were produced in the same manner as before described. The top row of doughs were
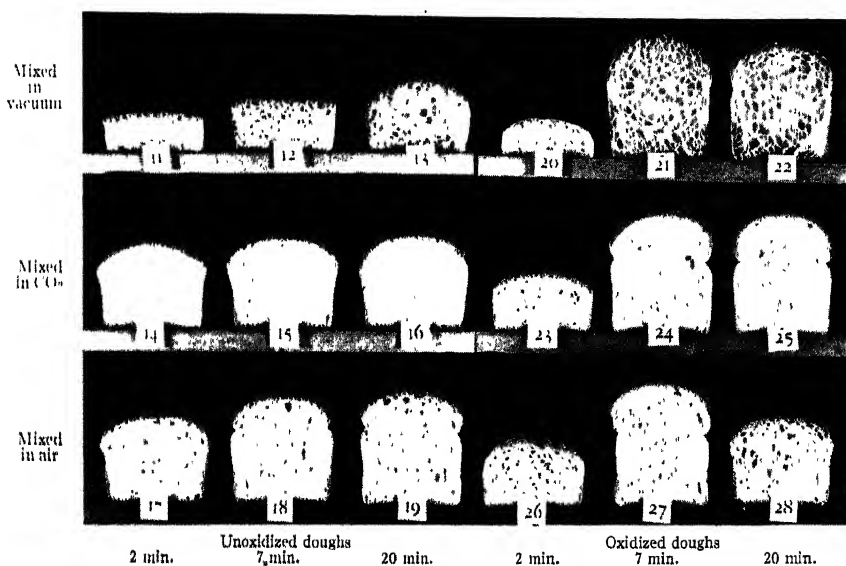
Fig. 7.   Effect of mixing time in vacuum, carbon dioxide, or air.

all produced in vacuum with mixing times of two, seven, and twenty minutes in each oxidized and unoxidized series. It is to be noted that with the unoxidized doughs the prolonged vacuum mixing has produced only a slight improvement in its quality. In no case is this unoxidized dough able to retain gas or give evidence of much cell structure in the finished bread. In the oxidized series a remarkable change occurs between the two minutes and seven minutes period of mixing. Though these six vacuum doughs were all of identical density coming from the mixer it is apparent that the short period of mixing has assembled the dough in such a manner that little gas can be re- -tained by the dough during the proofing and baking process, whereas

longer mixing renders the oxidized dough capable of retaining substantially all of the gas generated during proofing and bakes with a normal oven spring. It is also obvious that twenty minutes of mixing has substantially no additional effect upon the dough.

The middle shelf of breads in this figure were all produced by mixing in carbon dioxide; otherwise they were identical with the vacuum loaves immediately above them.

It is apparent that in the unoxidized dough very slight change is produced by altering the mixing time, all of the breads being of poor quality and typical of unoxidized no-time bread. Apparently the short period of mixing in this case has assembled the dough sufficiently to produce nearly as good bread as the long period of mixing. However, in the oxidized doughs a decided and pronounced effect is produced by increasing the length of mixing, a two-minute period being wholly inadequate to produce any appreciable retention of gas in this dough, whereas a seven-minute period has apparently assembled the dough in such a manner that the gases are retained. Longer mixing, up to twenty minutes, apparently has done no damage. It is now apparent that the reason the full seven-minute period is required to make optimum bread from this dough is that it produces an assembly of the dough in such a manner that the gas cells become gas-tight and retain the gases.

The bottom row of bread shows similar doughs mixed in air, the only difference here being that during the air mixing the oxygen has produced an improving effect in the unoxidized series and a possible improving effect for the seven-minute period in the oxidized series, followed by serious damage with further mixing.

In order to determine whether the difference between bread produced from unoxidized doughs as compared to bread produced from oxidized doughs is due to a fundamental difference in the manner in which gases emulsify with a resulting difference in the gas nuclei present in the dough, or whether the difference is caused by coalescence in the first instance and no coalescence in the other, a series of doughs (Figure 8) was prepared with increasing degrees of oxidation and baked by the regular procedure, resulting in the bread shown in the first column. It is to be noted that the increasing oxidation has produced marked improvement in the bread, followed by damage.

The second column shows the dough from which this bread was baked, frozen as it was ready to enter the oven after it had reached full proof. This picture was obtained by proofing the doughs in pup tins and immersing them in a dry-ice and acetone bath, which resulted in almost instantaneous freezing of the outer surface of the dough and complete freezing in a very short period of time. When freezing was

completed, the doughs were removed from the pans and a layer chopped off with a very sharp blade, exposing the cell structure of the rapidly frozen portion of the dough. This shows the condition of the dough at the time it enters the oven. The improvement produced in the bread by oxidation is apparent in the frozen dough.

Untreated

Slight oxidation

Optimum oxidation

Heavy oxidation

Baked bread full proof   Frozen dough full proof   Baked bread ¼ proof   Frozen dough ¼ proof
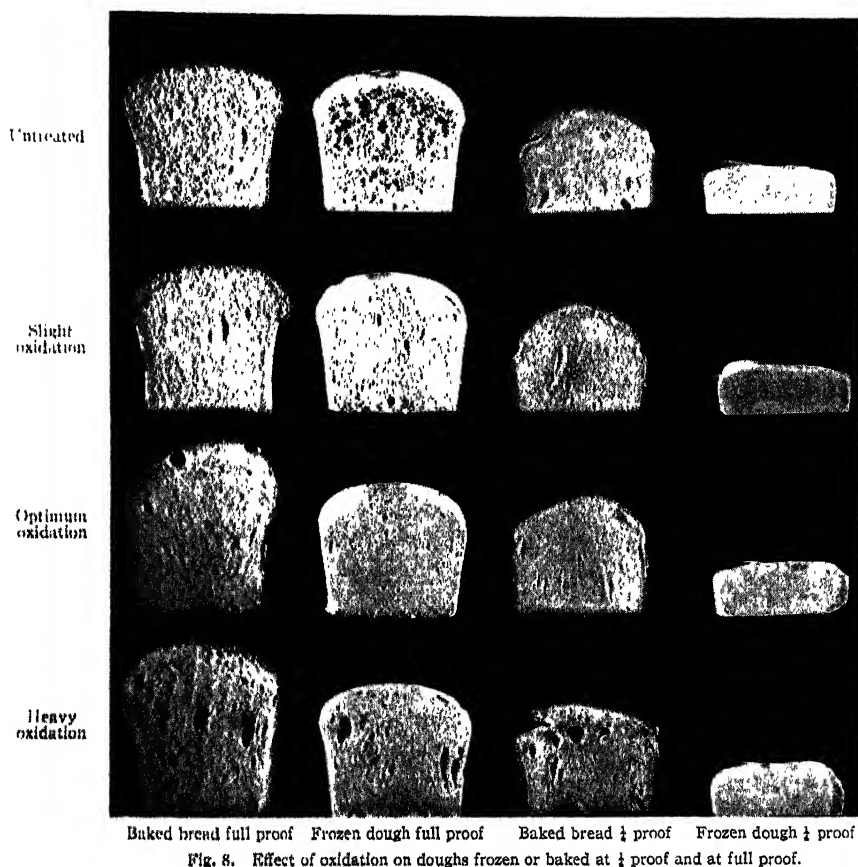
Fig. 8. Effect of oxidation on doughs frozen or baked at ¼ proof and at full proof.

Columns 3 and 4 (Figure 8) contain two similar series of breads and frozen doughs which were baked and frozen respectively after the first quarter of the proofing period had elapsed. It is now apparent that the frozen doughs are all identical and the baked breads are all substantially identical. One can conclude from this information that the number of gas-cell nuclei present in unoxidized dough at the end of the mixing period is substantially the same as the number present in oxidized dough. Also, one can conclude that the differences between

these two types of dough are due to the coalescence of the gas cells which occur during the proofing and baking period. In other words, the difference between these unoxidized and oxidized doughs is that in the former the cells are unable to expand sufficiently to accommodate the gas without breaking and joining together to make larger cells.

It is apparent by comparing the results in Figures 7 and 8 that the unoxidized doughs assemble quicker than do the oxidized doughs but they also coalesce more readily and lose their cell structure.

From the above experiments one can conclude that the gas nuclei in doughs can all be substantially produced by the beating-in action in the mixer and that the other sources of gas cells proposed at the beginning of this paper are either not needed or are without apparent effect upon the cell structure of the dough.

However, hypothesis No. 5—stretching, folding, punching, rolling, molding, and twisting—is one on which no information is shed by these experiments, for as planned, all of these actions upon the dough have been eliminated. It is already apparent that good textured bread can be produced and obtained without their use. The following experiments were performed to shed light upon the effect of these operations upon cell origin in bread making. However, it was not possible to retain the no-time dough technique as the operation of punching requires a period of fermentation. Therefore, subsequent experiments were made upon bread baked with a period of fermentation prior to panning.

Figure 9 shows effects of the molding operation upon the size of bread cells. All of the loaves shown in this chart were produced from oxidized doughs mixed in carbon dioxide in exactly the same manner as described above for no-time doughs. They were then put under 80 pounds pressure for five minutes to destroy all of the gas nuclei produced in the mixing operation. Loaf 1 was then extruded. Loaf 2 was passed through a machine molder immediately after releasing pressure. The subsequent loaves were molded after increasing periods of fermentation—Loaf 3 being molded after 20 minutes of fermentation, Loaf 4 after 40 minutes, Loaf 5 after 60 minutes. Loaf 6 was given a double molding operation, one at the end of 40 minutes and then again 20 minutes later. The loaves, after molding, were panned, proofed, and baked in the regular manner.

It is to be noted that the molder when used immediately after release of the pressure has added no cell structure as shown by Loaves 1 and 2, whereas, when the molder is used after the dough has had a period of fermentation to enable expansion to occur and the cells to grow in size before going to the molder, improved texture is obtained by molding, as shown in Loaf 3. The cell structure of this fermented

pressure dough before going to the molder must have been of very coarse nature, consisting of few bubbles, as shown by Loaf 1.

Loaf 3 shows that the character of cell structure has been modified by the stretching and rolling operation of the molder so that numerous smaller bubbles have been produced in the dough and are present in the bread. Apparently the molding operation had produced effects in the dough so that the number of gas cells was increased.

Loaf 4 shows that a greater increase in new cells was brought about when the dough was molded after having a 40-minute fermentation period.

However, Loaf 5 indicates that more fermentation up to 60 minutes had little further effect.

Extruded at once   Molded at once   Molded after 20 min.
      1   2   3

Molded after 40 min.   Molded after 60 min.   Molded twice in 60 min.
      4   5   6
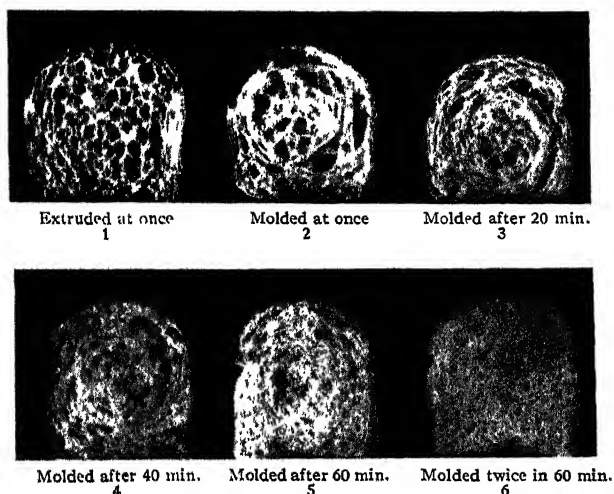
Fig. 9.   The effect of molding upon the size of bread cells.   Carbon-dioxide-mixed pressure doughs.

Loaf 6 indicates that if a dough which has been given the treatment received by Loaf 4—molding at the end of 40 minutes—is given a second fermentation period of 20 minutes, permitting a second expansion of the dough, and then another passing through the molder, texture is produced in the bread almost equal to that found in a normal loaf of bread. Here, then, is bread appearing normal in its texture characteristic which had substantially no gas nuclei present in it at the time it was removed from the pressure chamber. However, merely by passing such a dough twice through the molder at proper periods of expansion, good texture appears in the bread and a large number of gas cells have been produced in the dough by the act of going through the molder. It is apparent from this experiment

that in a molder the act of stretching and rolling a dough minutely subdivides the larger cells which were produced by the proofing.

Figure 10 pictures three exactly similar carbon-dioxide-mixed pressure doughs that were respectively baked, frozen, or molded at the end of 60 minutes' expansion after mixing. The purpose here is to show that both the baking and freezing of these pressure doughs disclose the same condition of the dough and also to show that the action of the molder has been to subdivide the cell structure of the dough.

Baked   Frozen   Molded and reproofed

Fig. 10. Cell structure of carbon-dioxide-mixed pressure dough prior to molding and baking.

Figure 11, picturing the relation of oxidation to molding, shows two series of bread. All loaves entered the oven $3\frac{3}{4}$ hours after mixing. In the upper series the proper amount of oxidation was used (5 ppm. of sodium chlorite) to produce optimum molded and punched bread in this period of time. The lower series are identical loaves each handled in exactly the same manner as the corresponding loaf directly above, with the exception that the amount of oxidation (30 ppm. of sodium chlorite) was the optimum required had the no-time technique been used.

The first pair of loaves in each row was mixed in vacuum for seven minutes. The last pair of loaves in each row was mixed in carbon dioxide for seven minutes. The carbon dioxide series and the vacuum series were handled otherwise exactly alike.

Loaves 1 and 4 were extruded directly into the baking pan from the vacuum mixer and allowed to stand in the pan $3\frac{3}{4}$ hours before baking.

Loaves 2 and 5 were identical to Loaves 1 and 4 but were put through a machine molder after $2\frac{1}{2}$ hours of fermentation, proofed $1\frac{1}{4}$ hours and baked.

It is to be noted that with light oxidation, the extruded dough mixed in vacuum coalesced and collapsed during the prolonged proofing and collapsed further during baking, as shown by Loaf 1. The corresponding heavily oxidized vacuum-mixed dough No. 4 in the second row continued to expand in the pan throughout the entire $3\frac{3}{4}$ hours' proofing period and at no time showed evidence of collapse. Neither did this dough collapse when placed in the oven, but gave a slight

additional oven spring. Apparently the heavy oxidation has produced a tough, expansible gluten structure which does not coalesce or collapse like the loaf immediately above. However, when these two doughs were passed through the machine molder at the end of the $2\frac{1}{2}$ hours' fermentation period and then proofed for normal proofing time, they both produced good bread but the loaf having the lesser degree of oxidation, No. 2, produced very much better bread than the strong-structured dough of heavy oxidation, loaf No. 5. Prior to passing through the molder, Loaf 2 was a flat, poor dough with apparently poor gas-retaining properties. However, upon being stretched and molded the resulting loaf shown was obtained. Apparently the action
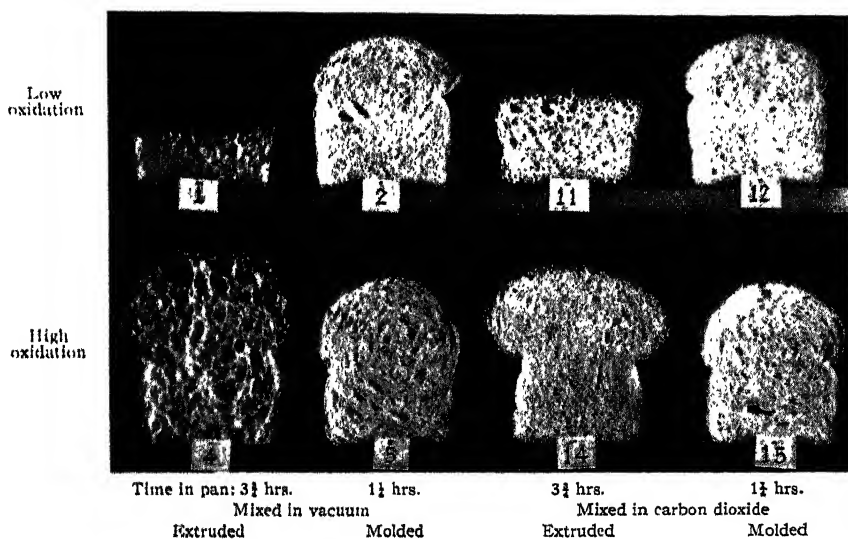
Fig. 11.   The relation of oxidation to molding.

of the molder in this case was to produce a greatly increased number of gas cells in the dough, which retained their identity through the normal proofing and baking period. However, in the heavily oxidized series the action of the molder gave no such fine gas-cell structure as was obtained in the case of the low oxidation, though the dough going to the molder possessed a much stronger and more voluminous gas structure. Apparently the molder was unable to produce from this gas structure as many fine cells as it was able to produce from the much softer, stickier, and more easily ruptured dough.

In the carbon-dioxide series of this chart Loaves 11 and 14 show a very similar action to Loaves 1 and 4. The dough with low oxidation was unable to retain the gas and a great amount of coalescence oc-

curred, whereas the heavily oxidized dough mixed in carbon dioxide also had a large volume but differed from the vacuum loaf, No. 4, in having a relatively fine cell structure. When these two doughs were put through the molder, the one with the low oxidation, Loaf 12, produced bread with very fine cell structure and good volume, as in the vacuum series, wheras the one with the high degree of oxidation, Loaf 15, suffered an actual loss in cell content. The molder was able to take the soft, relatively sticky, partially oxidized dough and create a relatively large number of gas cells, whereas it was unable to mold the heavily oxidized dough which still possessed many gas cells without destroying by its violent action a portion of this cell structure. Apparently the reason light oxidation is used with long fermentation is
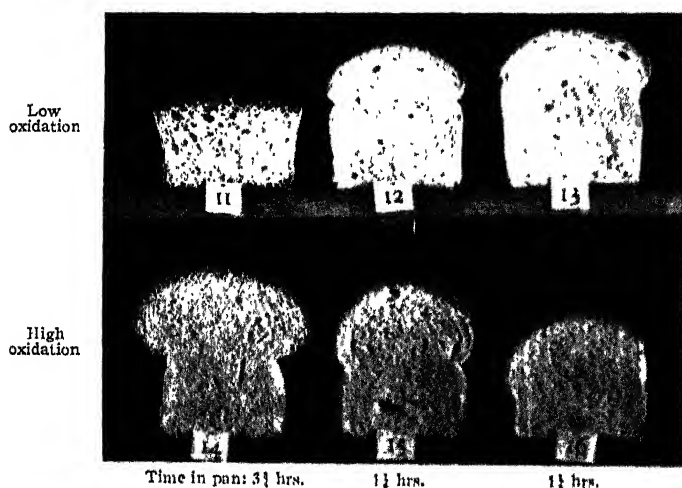
Fig. 12. The relation of oxidation to molding and punching.

because molding is capable of reviving and strengthening the cell structure. Where molding is applied immediately after mixing, prior to fermentation, heavy oxidation needs to be used to obtain desirable results. Molding of heavily oxidized dough after fermentation may cause destruction of the existing cell structure.

In order to study this problem further, Figure 12 contains the four carbon-dioxide-mixed doughs of the immediately previous figure with the additional Loaves 13 and 16, which were produced by punching the doughs twice during the fermentation period. In other respects they were handled identically to Loaves 12 and 15, which were molded only. It is now obvious that the punching operation has further improved the dough with a low degree of oxidation but it has very markedly

damaged the loaf with the high degree of oxidation. It is now apparent that the punching and molding operations of baking have beneficial action upon doughs which are sufficiently soft, sticky, and extensible for the operations to subdivide the existing gas cells. These same operations have a damaging effect upon doughs which are heavily oxidized.

Summary

The manner in which the gas cell in bread dough originates has been studied. Five hypothetical sources of the gas cell have been examined. For this purpose means were devised for making no-time bread of fine texture by using rapid oxidizing agents. An apparatus was built for extruding doughs so that the different sources of the gas cell's origin could be studied separately.

By mixing doughs in vacuum or by subjecting doughs to high pressure, the cells in the dough can be substantially eliminated. Their subsequent development can be used to show which of the sources of the gas cell are important.

It was thus shown that the yeast organism is incapable of originating gas cells in the dough itself. The entrained gases in the endosperm or occluded in the flour or beaten in during an early stage of mixing are of little or no consequence as a source of gas cells, in a properly developed dough.

The latter portion of the mixing period is capable of emulsifying all of the required gas to initiate the resulting gas cells which produce the texture in bread.

Punching and molding do not introduce any new gas cells into bread dough but create a greatly increased number of cells by subdividing those already present.

It is apparent that the texture in bread dough originates from those operations which apply work to the dough.

By quickly freezing dough just ready to go to the oven one can show that all of the cells found in bread exist in the dough when placed in the baking pan. The main differences in bread texture are due to coalescence and breaking of cells during molding, proofing, and baking.

The difference between oxidized and unoxidized doughs is not due to the amount of emulsification of which they are each capable. Rather they differ because an unoxidized or green dough does not have the capacity to prevent coalescence of bubbles during proofing and baking as does an oxidized dough. An overoxidized dough, on the other hand, is incapable of standing the severe action of punching and molding without breaking cells existing in such doughs.

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THE STRUCTURE OF THE GAS CELL IN BREAD DOUGH

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Fermenting bread dough, during its active expansion period, consists of a mass of gas bubbles which may be gas-tight. The bubbles have walls of dough that form an apparently gas-tight envelope. The structure and properties of the walls must of necessity determine the character of the dough.

Many studies of dough properties have been made and theories concerning bubble structure advanced. The wall of the dough bubble has been conceived of as a matrix of gluten and starch which, by their mutual adhesive properties, give it strength. Swanson and co-workers propound the theory that the properties of the wall structure are due to a glutinous network of strands. The gluten and starch structure is rendered gas-tight by the capillarity and surface properties of water which lies in and between these materials. In other theories the gluten is characterized as forming a continuous phase of protein material around the starch. The continuity and properties of the gluten phase in this theory determine the bubble's character.

This paper was initiated during a study of the dilution of dough by slowly adding 3% brine [1] during gentle stirring. Subsequent centrifuging of this diluted dough separates the ingredients into distinct layers. It was observed when unoxidized diluted doughs were centrifuged that the ingredients separated into three layers, namely starch at the bottom, gluten in the middle, and a briny layer on top. An oxidized dough, [2] when similarly diluted with 3% brine, developed a fourth layer above the brine. As the oxidation was increased the amount of this layer increased and became an appreciable proportion of the entire depth of the material in the tube.

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[1] Sufficient brine was used so that the total liquid added from the beginning was equal to two and one-half times the weight of the flour.
[2] Oxidation was accomplished by adding sodium chlorite up to 100 parts per million of the flour.

This top layer was carefully removed and diluted with more 3% brine. Mixture occurred readily and the material dispersed easily through the brine. When the admixture was made with only gentle stirring, so that the character of the material was retained as far as possible, it was noted that the layer separated into a glutinous foam that floated on the top and starch which settled by gravity to the bottom of the diluting liquid. This top layer could be removed and upon gently mixing in more 3% brine, would again separate to the top, appearing now like thin, translucent protein bubbles. These bubbles, which came from the original dough, were removed and examined for their properties. They were found to contain, on the anhydrous basis, approximately 45% protein. This bubbly material when placed in a vacuum chamber would expand to 10 volumes without breaking of the bubbles and, on release of the vacuum, would return to nearly its original condition. This expansion could be repeated more than once on the foam from the more highly oxidized doughs.

Apparently upon dilution of oxidized dough many of the smaller bubbles retain their integrity throughout the entire process. They are separated from the dough as such, and can be further washed to remove starch. Thus the material of which many dough-bubble walls is composed is shown to be essentially glutinous in character, containing approximately 45% protein and 55% fine starch. Apparently the bubbles which are separated from the dough depend upon a wall structure that is not dough in character.

A further series of observations was made on doughs fermenting in glass expansion jars. These present a bubble structure against the glass wall of the jar in such a manner that the interior surface of the bubbles can be observed. The walls of bubbles from unoxidized doughs are apparently dull and do not reflect light as would a soap bubble. Examination of the character of the bubbles in more highly oxidized doughs presents marked differences from those seen in unoxidized dough. Within the very small bubbles the surfaces are dull and not highly reflective but as the bubbles grow larger the surface becomes shiny and smooth with good reflection.

It is also to be noted that as these doughs reach a high degree of expansion there is apparent a new type of bubble having nearly transparent walls. Apparently some of the glutinous material has been able to free itself sufficiently from the starchy portion of the dough so that bubbles can be formed that are approximately transparent and the walls of which must of necessity be essentially glutinous in nature. Evidently during fermentation there is a tendency of the gluten to draw itself together away from the starch. In those bubbles which present a glossy surface the walls are probably glutinous at the surface

with relatively few of the larger starch granules in the surface to break up their reflective properties. Obviously one cannot collect such bubbles and determine their wall characteristics; neither could they be photographed.

When highly oxidized doughs are proofed to excessive heights and baked, a few of the films which constitute the bubble walls become sufficiently extended so that extremely thin, flat bread films can be collected and mounted. Good photomicrographs of the bread films were thus obtained with all parts sharply focused. The films were placed upon a microscope slide and covered with $n/100$ iodine solution. It was noted that the iodine solution did not color the bread film except along the edges where the film had been cut from the bread and on its surface only in spots where there were holes. The remaining surface of the film would not stain blue, though the iodine solution was removed with a blotter and repeatedly renewed. The bread film swelled in the solution, the aqueous portion of it penetrating through the surface of the bread film but apparently not carrying iodine into the interior, so that starch, if any, in this zone would stain. That starch did exist was definitely proved by scratching the surface of some of this film either before the iodine was applied or while it was in contact. A blue color would appear at the scratch.

It is apparent that these films of bread have walls of impervious glutinous material. That they were glutinous was shown by bathing with picric acid solution, upon which the walls stained a brilliant yellow color.

The accompanying photomicrographs (Figure 1) were taken of the bread film just described. They were taken to include the holes naturally occurring in the film and therefore show the manner in which the walls surrounding the dough bubbles puncture and fail, and thus permit gas to escape.

The upper left picture (No. 1) in Figure 1 shows a film magnified 75 diameters. The fine, wavy material is the glutinous wall stained yellow and the larger round dark objects are the starch granules stained blue. Only the starch surrounding the hole is stained. The picric acid solution has removed the blue from some of the more accessible starch grains. It is to be noted that the wavy material which constitutes the gluten wall of the membrane is continuous. This suggests again a gluten membrane which gives the bubble its gas holding properties. The wavy surface appearance is probably due to shrinkage of the film, after the puncturing of the bubble.

Photomicrograph No. 2 (Fig. 1) is a section of bread film with part of a larger hole, showing similarly a wall of glutinous substance and
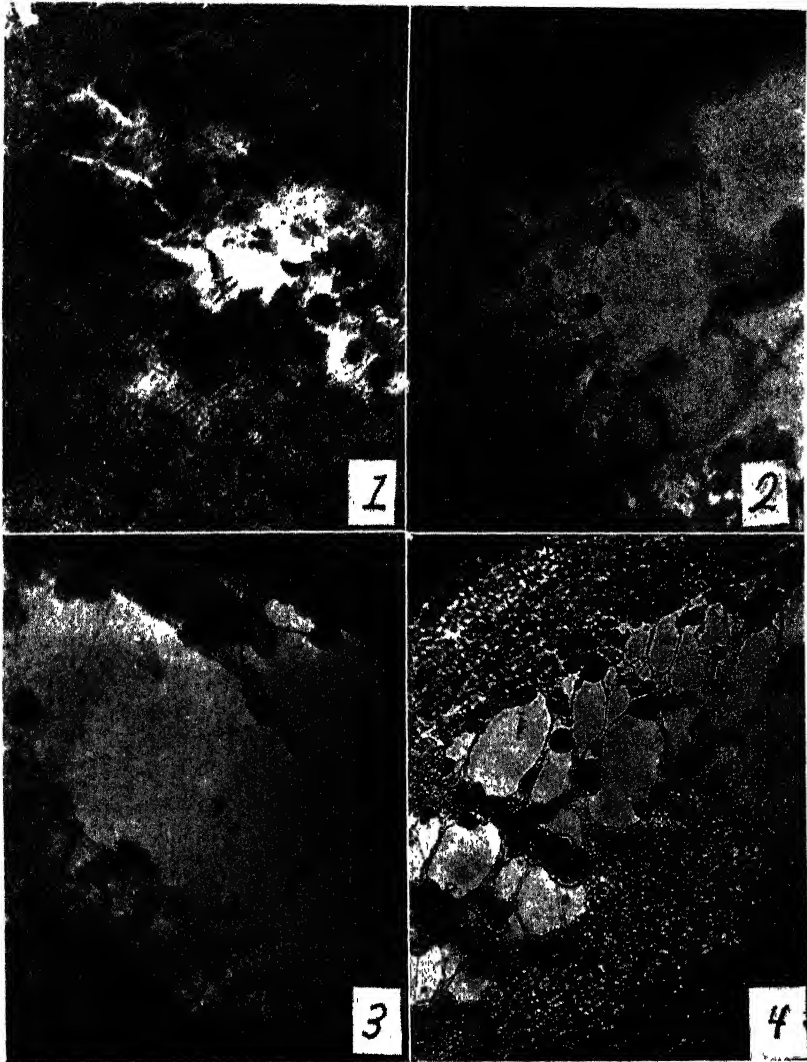
Fig. 1. Photomicrographs of films which form the cell walls in the crumb of overproofed bread. All pictures include ruptures produced in the film by escaping gas. Magnification 75 diameters.

starch particles as they lay in the bread material. This is clearly shown in the center section of the picture.

No. 3 is a similar picture, showing glutinous material pulling away from the film. The fluid character of gluten under the heat of baking becomes evident. It has stringy or fluid properties which permit it to draw out more or less like melted cheese. This is particularly well

shown in No. 4, where the fracture in the wall of the bread membrane is caught in the act of stretching and breaking. The expansion of the loaf must have ceased as the heat coagulated and set the melted proteins as shown. It is to be noted here that as the walls separated they pulled out into gelatinous or fluid strings to which the starch adheres. In many cases it can be noted, in this and the other pictures, that the glutinous material has an identity all its own and that the starch is not of necessity present in the gluten.

Further evidence supporting this theory is to be noted in the character of bread crumb. Bread made from unoxidized or green doughs has a dull appearance with very little sheen and stains easily with iodine, whereas quality bread is notably characterized by what the baker calls "sheen" or a very shiny appearance of the cell walls, and stains more slowly. Apparently unoxidized doughs are characterized by the presence of much starch in the wall, which roughens the surface, absorbs light, and prevents a shiny appearance, whereas in the properly developed doughs gluten is drawn into the surface of the cell wall and gives it a shiny appearance.

One can conclude from this work that the surface of a dough bubble may be either doughy in character, as in an unoxidized or green dough, or it may have a glutinous character, as in oxidized or developed dough.

These experiments suggest those properties which enable a gas bubble to retain its integrity while expanding. In oxidized or developed dough the gluten material has sufficient integrity or continuity so that it will not be torn apart but rather will draw more glutinous material into the expanding surface to supply the needs of the bubble and produce a gas-tight film on the inner surface of the bubble. The dough forms a matrix of glutinous and starch material from which the wall draws the required amount of gluten into its expanding surface by the strength of its elastic properties. In case the gluten does not have sufficient strength to accomplish this result it breaks and expansion of the bubble no longer continues. The properties of the bubble are largely determined by those properties of the gluten which enable it to retain its integrity as a film at the inner bubble surface. If the gluten itself has a relatively low viscosity and hence is somewhat fluid in character, it will break rather than pull more of the glutinous material into the film. Yet, in order to flow from the matrix the gluten must still be somewhat liquid in nature, but also tenacious. This tenacity is obtained because its viscosity is relatively high in comparison to its modulus of elasticity, as propounded by Halton and Scott Blair (1937), whereas the flow may be obtained because the numerical values of the viscosity and the elasticity are low.

A further condition in the dough that may affect the travel of gluten from the matrix into the surface film is affinity or adhesion between the starch granules and the flowing glutinous material. This affinity must be very much affected by the character of the starch granule surface. Thus may be explained the observation recently reported by Sandstedt, Jolitz, and Blish (1939), who describe the making of bread by recombining gluten and starch. They found that when the starch was treated with hypochlorite or chlorine the resulting bread was of very inferior quality to that which they made with unchlorinated starch. They also found that when they treated this chlorinated starch with α-amylase for a sufficient period of time the bread-making properties of the reconstituted starch-gluten dough were recovered. It is probable that an effect of chlorine, and later of α-amylase, is on the character of the surface. In view of the theory just expounded it is very possible that such alteration of the starch surface by causing a change in resistance to gluten flow may have a profound effect in determining the bread-making properties of dough.

Application of the Theory

It is evident from the experiments given that the wall of the gas bubbles in developed dough is essentially glutinous in character and that during the expansion of a bubble, gluten must necessarily be drawn to the surface by the expansion to supply the necessary wall to retain gases. It is also apparent that when a bubble which has a glutinous wall collapses, the surface material is drawn together and will produce a glutinous nucleus in the dough mass. It is then possible that the beneficial effect obtained upon dough by fermentation and the subsequent punching is due to the multiplying of such glutinous nuclei within the dough, providing the material for better bubble structure on subsequent expansion.

Speculations can be made concerning the mixing necessary to obtain dough development. The kind of mixing applied to doughs or used in forming them is important. Devices which rub, cut, or tear, damage rather than improve dough. They can only be used where their damage is later corrected during fermentation by punching or molding operations, or by subsequent proper mixing technique. Desirable mixing methods involve operations that stretch, fold, or squeeze the dough, such as are obtained in the Swanson or in the McDuffee mixer.

A dough-mixing machine was built which operated by rubbing dough between a revolving drum within a cylinder. A second device operated like a mixing conveyor, using many pins on both shaft and wall, and so operated that the chamber was kept filled with dough.

A third device was a small commercial laboratory batch dough mixer with a cover. When operated full of dough no stretching or folding took place. All of the above devices were incapable of mixing and developing dough so that even passable bread could be made under any condition of oxidation, speed, time of mixing, absorption, etc. There appears to be some magic in the stretching of dough which cannot be obtained otherwise.

It is well known that flour must be granular in order that good bread can be made. It is also well known in the industry that all mixing operations must be of the nature of stretching. In view of the conclusion here that a bubble requires a glutinous wall to function well, it would appear that the combination of granular endosperm particles plus stretching operations during the mixing may result in drawing glutinous material from the endosperm matrix and concentrate it between the surfaces of the endosperm particles. Such a result would provide ready-made material for gaseous nuclei with glutinous cell walls. The emulsed bubbles which are beaten into the dough can thus start to expand with initially formed glutinous walls.

With flour ground to a powder so that all the endosperm particles are disintegrated, such a phenomenon as classification of the gluten by mixing would appear difficult to accomplish. It is also apparent that overmixing of a dough would disintegrate the endosperm particles so that a result somewhat similar to overgrinding of flour might be obtained. Classification of the dough ingredients by further mixing would be rendered unlikely. However, fine grinding or overmixing, though making classification by mixing unlikely, would not preclude classification of gluten by bubble expansion and subsequent collapse of the bubble in punching, thus explaining some of the beneficial effects and corrective action obtained upon doughs by fermentation.

Summary

The washing out of bubbles from oxidized or developed bread doughs by careful dilution and rinsing methods has shown that these cells possess essentially thin glutinous walls which are capable of expansion in a similar manner to bread dough. Examination of the cell surfaces of a similar fermenting dough in glass jars shows the development of shiny surfaces. Later some of the bubbles develop transparent gluten walls. Similar observations of baked bread from oxidized or developed doughs shows them to possess shiny surfaces on the interior of the cell walls in contrast to the undeveloped doughs which possess bubbles of dull surface.

From greatly expanded bread doughs, thin, flat sections of bread films were obtained, stained, and photographed. By the manner in

which the stains were taken and the character of the ruptures shown in the film which were produced during the baking, one could note that they were covered with an impervious film of gluten on each surface and that starch was carried between the surfaces and could be stained by iodine only through points of rupture or damage.

These series of observations indicate that in a developed dough (particularly by means of oxidation) the bubbles acquire a thin film of gluten lining the surface which gives the bubble its shiny appearance and must contribute greatly to its gas-tight properties and strength. It is conceived that this film is drawn to the surface because the gas nucleus from which the bubble originated started in a glutinous core. As the bubble was expanding the required amount of gluten to satisfy its surface needs was drawn from the starch-gluten matrix of the endosperm material. The properties that enabled this to occur may be controlled by the viscosity and fluidity of the gluten and by the amount of adhesion of the gluten to starch.

These experiments suggest that the beneficial effects obtained from punching doughs is due to the collapsing of such glutinous cell surfaces during the punching, thereby providing glutinous centers in which the contained gaseous nuclei can develop with subsequent tighter gluten walls.

It has been shown that the mixing develops dough by means of stretching and folding which occur during the mixing operation. It is conceived that the stretching operation thereby draws gluten from the endosperm particle matrix to the surface of the particle and produces there gluten concentrate, which is available for holding gas nuclei produced in the mixing, thus partially explaining the development which is acquired during the mixing period.

Acknowledgment

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THE EFFECT OF WHEAT TYPE, PROTEIN CONTENT, AND MALTING CONDITIONS ON THE PROPERTIES OF MALTED WHEAT FLOUR [1]

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In recent years the production of malted wheat flour for use in increasing amylase activity of flour deficient in this respect has assumed wide commercial importance. There is, however, little published information on the effects of variety, environment, and malting conditions on the properties of wheat malt, although many investigations have been made of the effects of these factors on barley malt.

Early work on the germination of wheat, which has been reviewed by Bailey (1925), was largely confined to studies of the effect of germination time. In general, these studies revealed that the extent of germination must be carefully controlled in order to avoid undesirable effects. In later investigations Sherwood and Bailey (1926a, 1926b) confirmed and extended these observations and suggested that germination periods longer than three days were unsuitable, probably because of excessive proteolytic activity in malts produced under these conditions. The importance of short germination and minimum development of proteolytic enzymes is also stressed in a patent issued to Schreier (1934).

Since the publication of these papers, experimental malting equipment has been developed, primarily for use with barley, which permits of precise control and investigation of steeping, germination, and kilning conditions. With such equipment available, it seemed desirable to reinvestigate the effect of germination time on the properties of malted wheat and to study the importance of additional factors. Accordingly the present study was designed to obtain preliminary information on the effect of type of wheat, protein content, steeping level, germination time, and kilning temperature on the value of the malt for diastating purposes. In order that clear-cut results might be obtained with a relatively small investigation, type was represented by hard red spring and durum wheats and each of the other variables was studied at two widely different levels. A factorial design was thus obtained in which samples

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of high- and low-protein hard red spring and durum wheats were malted by each of eight methods. The 32 malts were milled, and the amylase and protease activities of the resulting flours and the effects on baking quality of adding them to a low-diastatic flour, were studied.

Experimental

Materials.—Two samples of Western Canadian hard red spring wheat of 14.3% and 12.1% protein content and two samples of Western Canadian amber durum wheat of 14.2% and 12.5% protein were employed. These were obtained by compositing residual lots of No. 1 Northern and No. 1 Canada Western grades from the 1937 protein survey collections of the Dominion Grain Research Laboratory. The range in protein content of the subsamples in each series did not exceed 1%. Thus it will be seen that the samples comprised two widely different types of wheat at two protein levels differing by approximately 2% and were fully representative of wheats available in commercial quantities.

Malting.—Eight malts were made from each sample of wheat using eight different treatments, representing the combination of steeping to 44% and 40% moisture content, germinating for 5 and 3 days, and kilning for 12 hours at 100° F. and for 12 hours with the temperature rising steadily from 100° to 130° F. The eight treatments may be conveniently designated by numbers of three digits, the first representing the steeping procedure, the second the germination procedure, and the third the kilning procedure, as shown in the first four columns of Table I.

The available germination equipment consisted of two chambers (Anderson, 1937), each accommodating eight samples. It was therefore necessary to make two batches, each comprising 16 malts. Possible differences between the malts made in different batches were offset in part by confounding the triple interaction between steeping, germination, and kilning with the batch error. Thus treatments 112, 211, 121, and 222 were used in the first batch, and the remaining treatments were used in the second batch.

The malts which were to be germinated for three days were started two days after starting the malts which were to be germinated for five days. In consequence all malts in one batch were ready for kilning at the same time. Two kilns, each holding eight samples, were available, and one was used for each kilning treatment.

Aliquot parts of the wheat samples, representing 350 g. of dry matter, were steeped in quart jars immersed in a water bath controlled at 50° F. At the end of each 11 hours the samples were aerated by

draining off the water and allowing the closed jars to float in the water bath for one hour, after which the jars were filled with fresh water. The times required for each sample to attain a moisture content of 44% and 40% were determined by pilot experiments and proved to be 48 and 24 hours for the high-protein samples and 56 and 30 hours for the low-protein samples.

The wheats were germinated in the equipment described by Anderson (1937) in cylindrical galvanized iron cans (6 × 6 inches) containing 200 $\frac{1}{8}$-inch holes. The temperature of the germination chambers was maintained at 54° F. The malts generate considerable heat and their temperature is thus raised 3° to 5° F. above that of the chamber. The green malts were dried in the kiln described in the same paper, using cylindrical 8-mesh wire cages, and the temperatures noted above.

Immediately after kilning the malt was kneaded in a small bag. The sprouts which were rubbed off during this process were separated by sifting and weighed to the nearest 0.1 g. The polished malt was weighed to the nearest 0.5 g. and its dry-matter content subsequently determined in the vacuum oven. Malt yield and sprouts are reported as grams per 100 g. of wheat dry matter.

Owing to a combination of circumstances beyond the control of the authors a period of 18 months elapsed between the making of the malts and their subsequent use. During this period they were kept in air-tight tins at approximately 2° C. While the enzymic activity of these samples may have decreased as a result of such storage, nevertheless it is felt that such changes should not materially affect the validity of comparisons between the different malts. It would normally be expected that storage would tend to decrease absolute differences between samples, but since the variables investigated were studied at widely separated levels, any appreciable effect of any single factor should be apparent.

Milling.—The milling was carried out in a micro mill similar to that described by Geddes and Frisell (1935). The malts were tempered to 12.5% moisture content and allowed to stand 24 hours. With durum malts sufficient water to raise the moisture content to 13.5% was added one hour before milling. In the instance of spring wheat malts this addition was made 30 minutes prior to milling. Samples were milled to a constant yield of 50% malt flour passing a 10XX silk.

As an index of the replicability of the milling technique, nine aliquot portions of a bulk lot of malted wheat were ground by the above procedure and ash and amylase activity of each malt flour were determined. The latter property was evaluated by adding each malt flour to a common base flour at a level of 0.25% and determining the diastatic activity by the Blish and Sandstedt (1933) method as modified by Sandstedt (1937). The respective mean values were 0.363% and 207.0 mg. mal-

tose per 10 g.; in both cases the variation between malts was not significantly greater than the corresponding variation between duplicates. Accordingly, it was assumed that variability in milling technique would not constitute an appreciable source of error.

Analytical methods.—The moisture, ash, and protein content of the experimental material were determined by standard A. A. C. C. methods, and proteolytic activity by the method of Ayre and Anderson (1939). It has been shown by Hildebrand (1940) that this procedure gives results with malted wheat flours which are highly correlated with those obtained by the Landis and Frey (1938) rate-of-gelation method. It has been assumed that both methods afford an estimation of proteinase activity and are little affected by the presence of other types of proteolytic enzymes. These methods therefore should yield results which are of more significance in relation to dough protein structure than procedures which also measure dipeptidase and polypeptidase action.

Amylase activity was estimated by the technique described by Davis and Tremain (1938), as modified by Hildebrand and Geddes (1940). In brief, this procedure is based upon the addition of increments of malt flour to a common base flour, determination of the gassing power and/or diastatic activity of the resulting blends, and calculation of the amount of malt necessary to produce an arbitrarily selected level of either measure. Amylase activity was conveniently expressed as the reciprocal of the dosage thus calculated. In this study, such amylase values were obtained from both gassing-power and diastatic-activity measurements.

Baking procedure.—All flours were baked by a modification of the A. A. C. C. basic procedure, using 5% sugar, mixing for 2 minutes in the Hobart-Swanson mixer, and fermenting for 4 hours. ·Doughs were punched and moulded with the aid of a National dough sheeter.

Growth and Yield of Malt

If different malting equipment were used it might well prove difficult to obtain malts closely similar to those used in the present study merely by following the malting procedures previously described. In these circumstances it seems advisable to describe the malts not only in terms of the conditions under which they were made but also in terms of growth and yield. The necessary data are summarized in Table I as means for each treatment over all samples.

The different treatments produced malts representing a fairly wide range in growth of roots and acrospires and in yield of roots and dressed malt. Minimum growth was obtained with treatment 222 in which only one root with a length equal to 0.8 of the kernel was formed; the acrospire was only just showing and the yield of malt was 98% of the

TABLE I

AVERAGE EFFECT OF TREATMENTS ON GROWTH OF ROOTS
AND ACROSPIRE, AND ON MALT YIELD

No.	Malting treatment			Length compared to kernel length		Moisture content of malt	Yield (dry basis)	
	Steep.	Germ.	Kiln.	First root	Acro-spire		Roots	Dressed malt
	Moisture							
	%	Days	°F.			%	%	%
111	44	5	100	2.0	0.7	10.2	3.8	93.4
112	44	5	100–130	1.8	0.7	8.1	3.8	93.0
211	40	5	100	1.4	0.5	9.9	1.8	96.3
212	40	5	100–130	1.6	0.5	8.2	1.6	96.3
121	44	3	100	1.1	0.4	10.1	1.7	96.6
122	44	3	100–130	1.4	0.4	8.3	1.7	96.3
221	40	3	100	1.0	Just showing	9.8	0.7	98.1
222	40	3	100–130	0.8	Just showing	8.1	0.7	98.0

weight of the original wheat. Maximum growth was obtained with treatment 112 in which three roots were formed, the longest being 1.8 times the length of the kernel; the acrospire grew to a length equal to 0.7 times that of the kernel and the yield of malt was 93%. The remaining treatments produced intermediate results.

TABLE II

MEAN DATA ON GROWTH AND YIELD OF MALT FOR EACH WHEAT SAMPLE

Sample	Protein content of wheat (13.5% m.b.)	Length compared to kernel length		Moisture content of malt	Yield (dry basis)	
		First root	Acro-spire		Roots	Dressed malt
	%			%	%	%
Hard red spring	14.3	1.4	0.4	9.1	2.0	96.1
Hard red spring	12.1	1.4	0.4	9.2	1.9	96.1
Amber durum	14.2	1.4	0.4	9.0	2.1	95.9
Amber durum	12.5	1.4	0.4	9.1	2.0	95.9

The mean data for each wheat sample, over all treatments, are given in Table II. Inspection of the data will show that, on the average, all four samples gave almost identical results.

Table III shows the average effect, over all samples, of changing the steeping, germination, and kilning treatment. Increasing the steeping and germination time had the expected results: growth rate and yield of roots tended to increase and yield of malt was decreased. Increasing

TABLE III

AVERAGE EFFECT OF CHANGES IN STEEPING, GERMINATION, AND
KILNING TREATMENTS ON GROWTH AND MALT YIELD

Effect of increasing:	Length compared to kernel length		Moisture content of malt	Yield	
	First root	Acro-spire		Roots	Dressed malt
			%	%	%
Steeping level	0.4	0	0.2	1.6	−2.4
Germination time	0.6	0.4	0	1.4	−2.4
Kilning temperature	0	0	−1.8	0	−0.2

the kilning temperature reduced the moisture content of the malt and reduced the yield of malt slightly. This last result suggests that respiration continued during the earlier stages of kilning and was more rapid at the higher temperature.

Protein and Ash Content of Malted Wheats and Flours

The average effect of malting conditions on protein and ash content is shown in Table IV and comparable data for the different wheat samples are reported in Table V. In order to facilitate comparisons, the

TABLE IV

AVERAGE EFFECT OF MALTING TREATMENTS ON PROTEIN AND ASH
CONTENT OF MALTED WHEAT AND MALT FLOUR [1]

No.	Malting treatment			Wheat		Flour	
	Steep.	Germ.	Kiln.	Protein	Ash	Protein	Ash
	Moisture, %	Days	°F.	%	%	%	%
111	44	5	100	13.25	1.530	10.75	0.445
112	44	5	100–130	13.22	1.542	10.72	0.440
211	40	5	100	13.35	1.565	11.02	0.452
212	40	5	100–130	13.48	1.560	11.00	0.440
121	44	3	100	13.48	1.558	11.15	0.478
122	44	3	100–130	13.48	1.522	11.18	0.440
221	40	3	100	13.45	1.648	11.32	0.452
222	40	3	100–130	13.62	1.602	11.42	0.450

[1] Protein and ash results are expressed on a 13.5% moisture basis.

data of Table IV are summarized in Table VI, in which only the statistically significant differences are given. The comparisons in Table VI indicate that conditions which tended to increase growth lowered protein content. This is a natural effect of increased germinative activity

TABLE V

Mean Data on Protein and Ash Content for Each Wheat Sample [1]

Sample	Original wheat	Malted wheat		Malted flour	
	Protein	Protein	Ash	Protein	Ash
	%	%	%	%	%
Hard red spring	14.3	14.38	1.320	11.89	0.332
Hard red spring	12.1	12.08	1.668	9.51	0.388
Amber durum	14.2	14.24	1.664	12.41	0.604
Amber durum	12.8	12.98	1.612	10.48	0.475

[1] Protein and ash results are expressed on a 13.5% moisture basis.

TABLE VI

Average Effect of Changes in Steeping, Germination, and Kilning Treatments on Malted Wheat and Flour Protein and Ash

Effect of increasing:	Wheat		Flour	
	Protein	Ash	Protein	Ash
	%	%	%	%
Steeping level	−0.12	−0.056	−0.24	
Germination time	−0.19	——	−0.39	
Kilning temperature	——	——		

resulting in greater translocation of nitrogenous materials to the developing roots and acrospires, which were largely removed in dressing the malt. Presumably the decreases registered would be greater if it were not that increased translocation is accompanied by increased respiratory activity. The latter process takes place at the expense of carbohydrates and therefore would tend to increase the percentage of other constituents. The decrease in protein content is greater in the instance of the flours than of the wheats, since translocational activity originates in the endosperm.

In general, decreases in ash content as a result of translocation were too small to be significant.

Amylase Activity

In the measurement of amylase activity, the malted wheat flours were added to a low-diastatic (165 mg. maltose per 10 g. flour) Southwestern patent flour in increments of 0.25, 0.50, 0.75, and 1.0%. In the instance of twelve of the malts, which were found to be low in activity, a 2.5% addition was also made. Diastatic activity was determined in duplicate on this series of diastated flours by the Blish and Sandstedt (1933) method as modified by Sandstedt (1937). Gassing power was

determined as outlined by Sherwood, Hildebrand, and McClellan (1940), the total gas production at 30° C. for five hours being recorded.

Arbitrary levels of 280 mg. maltose per 10 g. of flour and the corresponding level of 204.7 ml. gas production were selected and the amounts of malted wheat flour required to attain these levels were computed as described by Hildebrand and Geddes (1940). The relative amylase activities of the malted wheat flours were expressed as the reciprocals of these values in order that the resulting figures would bear a direct rather than an inverse relation to activity. A statistical analysis of the values thus computed showed that there was no significant difference in amylase-activity values obtained from diastatic activity and gassing power determinations. Accordingly, the mean values for both methods combined were taken as the best estimate of activity for the purposes of this investigation.

The mean effects of the malting treatments over all samples are recorded in Table VII, and for each sample over all treatments in Table VIII. The average effects of all variables on amylase activity which were found to be statistically significant are shown in Table IX. The effect of any one variable, however, was in some cases conditioned by the

TABLE VII

AVERAGE EFFECT OF MALTING TREATMENTS ON AMYLASE AND PROTEASE ACTIVITY OF MALTED WHEAT FLOURS

No.	Steep moisture	Germination	Kilning temp.	Amylase activity	Protease activity
	%	Days	°F.	Units	Units
111	44	5	100	3.78	201.0
112	44	5	100–130	3.80	201.8
211	40	5	100	2.70	111.8
212	40	5	100–130	2.87	104.4
121	44	3	100	1.06	106.0
122	44	3	100–130	0.89	104.8
221	40	3	100	0.73	80.0
222	40	3	100–130	0.70	75.6

TABLE VIII

MEAN AMYLASE AND PROTEASE ACTIVITY FOR EACH WHEAT SAMPLE

Sample	Original wheat protein content	Amylase activity	Protease activity
	%	Units	Units
Hard red spring	14.3	2.37	127.7
Hard red spring	12.1	2.07	84.5
Amber durum	14.2	1.88	154.2
Amber durum	12.8	1.94	126.2

TABLE IX

Effect of:	Amylase activity	Protease activity
	Units	*Units*
Wheat type (hard red spring minus durum)	0.31	−34.1
Increasing protein content	----	35.5
Increasing steeping level	0.64	60.5
Increasing germination time	2.44	63.1
Increasing kilning temperature	—	—

level of one or more of the other variables involved. Thus for example the effect of protein content depended upon the type of wheat and upon the steeping conditions. Where such significant interactions were found, the pertinent means are given in Table X.

The mean value for both protein levels and all malting treatments for malt flours produced from hard red spring wheat was higher than the corresponding value for durum malt flours. However, the difference between these wheat types varied with both protein level and malting conditions. Thus the difference was significantly greater at the higher protein level, with the shorter steeping, and at the five-day germination.

The effect of variation in protein content was not significant, considering both types of wheat over all malting conditions. Differences due to protein were found but they were not consistent. Thus with hard red spring wheat malts the high-protein samples had the higher activity, while with the durum malts the difference was much smaller and in the reverse order. The same sort of effect was observed with respect to the interaction of protein content and steeping time.

Considering now the influence of malting conditions on amylase activity, the greatest effect was that of germination time; in fact this single variable was responsible for over 80% of the total variability encountered. The malts grown for five days showed markedly higher activity for both wheat types at both protein levels and under all conditions of steeping and kilning. There were, however, as shown in Table X, two significant interactions involving germination time, namely those with wheat type and with steeping level. For the hard red spring malts the difference between the five- and three-day germinations was greater than the comparable difference for the durum malts, although this differential response was small compared to the total effect of germination time.

The interaction between steeping and germination represents a somewhat different situation from the interactions previously discussed. As in all interactions, the absolute effect of one factor is conditioned by

TABLE X
Significant Interactions—Amylase and Protease Activity

	Amylase Activity					
	Wheat type and:					
	Protein		Steeping		Germination	
	High	Low	44%	40%	5 days	3 days
Hard red spring	2.37	2.07	2.43	2.01	3.69	0.76
Amber durum	1.88	1.94	2.33	1.49	2.89	0.93
Difference	0.49	0.13	0.10	0.52	0.80	−0.17

	Protein and:				
	Wheat type			Steeping	
	Spring	Durum		44%	40%
High protein	2.37	1.88		2.53	1.71
Low protein	2.07	1.94		2.23	1.78
Difference	0.30	−0.06		0.30	−0.07

	Germination and:				
	Wheat type			Steeping	
	Spring	Durum		44%	40%
Five days	3.69	2.89		3.79	2.78
Three days	0.76	0.93		0.97	0.71
Difference	2.93	1.96		2.82	2.07

	Steeping and:					
	Wheat type		Protein		Germination	
	Spring	Durum	High	Low	5 days	3 days
Steeping to 44%	2.43	2.33	2.53	2.23	3.79	0.97
Steeping to 40%	2.01	1.49	1.71	1.78	2.78	0.71
Difference	0.42	0.84	0.82	0.45	1.01	0.26

	Protease Activity		
	Protein and:		
	Steeping		
	to 44%	to 40%	Difference
High protein	180.3	101.6	78.7
Low protein	126.5	84.3	42.2
Difference	53.8	17.3	—

	Steeping and:		
	Germination		
	5 days	3 days	Difference
Steeping to 44%	201.4	105.4	96.0
Steeping to 40%	108.1	77.8	30.3
Difference	93.3	27.6	—

the level of the other. In this instance, however, the ratios between activities for the long and short germination times are virtually identical for both steeping levels. In both cases, decreasing germination from five to three days reduced the activity to 25.6% of its original value. In like manner reducing steeping level brought about a lowering of activity to 73.3% of the original value under both germination conditions. In other words, the activity for three-days germination at 40% steeping level was exactly equal to the value calculated from the combined application of the separate effects of germination and steeping. Thus $3.79 \times 0.733 \times 0.256 = 0.71$. It is therefore clear that this interaction in no sense represents an anomalous effect.

With the exception of germination time, the effect of steeping treatment was of the most significance, the higher steeping producing the greater amylase activity. This difference existed for both wheats, at both protein levels, and for the different germination and kilning treatments, but its magnitude was not consistent throughout. As may be seen from Table X, the effect of steeping was greater in the instance of durum wheat, at the high-protein levels and for the longer germination time. It has already been shown that the interaction between steeping level and germination time agrees with the calculated effects of the simultanous application of both factors.

Variation in kilning treatment within the limits tested was without influence on the amylase activity of the malts.

Protease Activity

The protease activity values are also recorded in Tables VII to IX and the significant interactions in Table X. The durum wheat malt flours were consistently and markedly higher in proteolytic activity than the spring wheat malt flours; with regard to the effect of protein, the high-protein samples gave the higher average protease activity but the difference was not entirely consistent. Thus with wheats steeped to 44% moisture, the high-protein malt flours gave protease values averaging 54 units higher than the low-protein malts, whereas in the instance of wheats steeped to 40% moisture the difference was only 17 units. There were no significant interactions of other variables with protein level.

With regard to the effect of malting conditions, steeping and germination treatments both had a pronounced and approximately equal effect on protease activity. Steeping to the higher level in all cases increased protease activity. However, as is shown in Table X, the response to changes in steeping treatment was conditioned by both protein and germination level. Increasing germination time resulted in higher

protease activity. This effect was consistent except under different steeping conditions as noted above; here the difference was greater when the samples were steeped to 44% moisture. Differences in kilning treatment were consistently without measurable effect.

Relation between Amylase and Protease Activity

The amylase and protease activities of the various malt samples are shown in Table XI. The correlation between these variables for all samples was + .676, a highly significant value since the corresponding 1% point is 0.449. However, the correlation accounted for only 46% of the total variability and inspection of the data in Table XI indicates that the association between amylase and protease activity was markedly affected by the various factors under investigation.

TABLE XI

MEAN VALUES FOR AMYLASE AND PROTEASE ACTIVITIES OF MALTED WHEAT FLOURS

	Hard red spring				Amber durum			
	High protein		Low protein		High protein		Low protein	
Treatment	Amy-lase	Pro-tease	Amy-lase	Pro-tease	Amy-lase	Pro-tease	Amy-lase	Pro-tease
	Units	Units	Units	Units	Units	Units	Units	Units
111	4.53	231	3.43	150	3.40	246	3.76	177
112	4.52	205	3.72	168	3.39	228	3.56	206
211	3.18	113	3.28	66	2.11	130	2.22	138
212	3.35	108	3.48	60	2.16	113	2.48	136
121	1.15	122	0.65	72	1.32	144	1.12	86
122	0.82	115	0.66	69	1.14	151	0.94	84
221	0.74	65	0.74	49	0.77	123	0.66	83
222	0.64	62	0.65	42	0.74	98	0.75	100
Mean all treatments	2.367	127.7	2.074	84.5	1.880	154.2	1.938	126.3

The most practical method of evaluating the relationship between these two types of activity seemed to be to study the variation in protease activity of quantities of malt so selected as to give a constant level of amylase activity. Accordingly, the dosage of each malt required to produce a constant level of gassing power (204.7 ml. in 5 hours at 30° C. per 14 g. flour with 3% yeast) was calculated. This gassing-power level with the base flour employed was found to be equivalent to 280 mg. maltose per 10 g. flour, as determined by the Blish and Sandstedt method. These calculated dosages are given in the second column of Table XII.

In order to test the accuracy of this technique, these amounts of malt flour were added to the same base flour used throughout the investigation and duplicate determinations of gassing power were made. The mean value for all blends was 205.5 ml. and the variation between blends was not significantly greater than the duplicate error.

TABLE XII
Malt Dosages to Give Constant Gassing Power and Protease Activity Units Contributed

Malting treatment	Malt dosage to give 204.7 ml. CO_2, %	Protease activity	
		Units per 100 g. malt	Units contributed by malt at constant gassing power
HARD RED SPRING—HIGH PROTEIN			
111	0.229	231	0.53
112	0.246	205	0.50
211	0.352	113	0.40
212	0.298	109	0.32
121	0.879	122	1.07
122	1.171	115	1.35
221	1.398	65	0.91
222	1.552	62	0.96
HARD RED SPRING—LOW PROTEIN			
111	0.302	150	0.45
112	0.304	168	0.51
211	0.310	66	0.20
212	0.282	60	0.17
121	1.530	72	1.10
122	1.538	69	1.06
221	1.354	49	0.66
222	1.588	42	0.67
AMBER DURUM—HIGH PROTEIN			
111	0.318	246	0.78
112	0.314	228	0.72
211	0.462	130	0.60
212	0.492	113	0.56
121	0.770	144	1.11
122	0.870	151	1.31
221	1.298	123	1.60
222	1.308	98	1.28
AMBER DURUM—LOW PROTEIN			
111	0.302	177	0.53
112	0.290	206	0.60
211	0.450	138	0.62
212	0.398	136	0.54
121	0.932	86	0.80
122	1.037	84	0.87
221	1.392	83	1.16
222	1.382	100	1.38

The third column of Table XII shows the mean protease activities of each malt and the last column shows the calculated protease activity contributed by the quantities of malt required to produce the constant level of gassing power referred to above.

The average effect of malting treatments for the four wheats on protease activity at constant amylase activity is given in Table XIII and the

TABLE XIII

AVERAGE EFFECT OF STEEPING, GERMINATION, AND KILNING TREATMENTS
ON PROTEASE ACTIVITY AT CONSTANT AMYLASE ACTIVITY

Treatment	Steeping moisture	Germination	Kilning temp.	Protease activity
	%	Days	°F.	Units
111	44	5	100	0.572
112	44	5	100–130	0.582
211	40	5	100	0.455
212	40	5	100–130	0.398
121	44	3	100	1.020
122	44	3	100–130	1.148
221	40	3	100	1.082
222	40	3	100–130	1.072

TABLE XIV

AVERAGE EFFECT OF WHEAT TYPE AND PROTEIN CONTENT ON PROTEASE
ACTIVITY AT CONSTANT AMYLASE ACTIVITY

Wheat type	Wheat protein	Protease activity
	%	Units
Hard red spring	14.3	0.755
Hard red spring	12.1	0.602
Amber durum	14.2	0.995
Amber durum	12.8	0.812

TABLE XV

AVERAGE EFFECT OF WHEAT TYPE, PROTEIN CONTENT, AND MALTING CONDITIONS
ON PROTEASE ACTIVITY AT CONSTANT AMYLASE ACTIVITY

Effect of:	Difference in protease activity
	Units
Wheat type (hard red spring minus amber durum)	−0.225
Increasing protein content	0.168
Increasing steeping level	
a. Hard red spring	0.285
b. Amber durum	−0.128
Increasing germination time	−0.579
Increasing kilning temperature	—

corresponding values for the effect of wheat type and protein content in Table XIV. In order to show these effects more clearly the differences between the two levels of each factor are given in Table XV, only statistically significant values being shown. From these comparisons, a lower level of protease activity for a given level of gassing power would result from the use of low-protein hard red spring wheat steeped to 40% moisture and germinated for five days.

Baking Tests

In order to ascertain whether the variations in proteolytic activity discussed above, or other undetermined factors, would affect the baking characteristics of flours diastated with the various malts, blends were prepared using the quantities of the different malt flours shown in Table XII. Thus a series of flour blends of equal gassing power, as proved by actual test, was obtained. These blends were baked by the technique previously outlined, which involved the use of 5% sugar and a four-hour fermentation time. A fermentation time longer than that of the A. A. C. C. basic procedure was used to provide a greater opportunity for detecting the effect of variations in protease activity or other factors. In order to preclude the possibility that a deficiency in gassing power might influence the results under the longer fermentation conditions, 5% sucrose was added. The flours were baked in triplicate in random order.

The mean loaf volume for all flours and replicates was 586.0 cc. The variation within replicates was not large (standard error of single determination = 20.2 cc.) yet the variation between flours was less than 10% of this replicate variability. Obviously, therefore, the variations in proteolytic activity were without influence on loaf volume. No differences in external or internal characteristics of the bread or in the dough handling properties were detectable and it may therefore be concluded that under the conditions of these experiments, factors other than amylase activity were without appreciable influence on the value of the malt for diastating purposes.

Discussion

This project was undertaken primarily to secure an estimate of the relative importance of the various factors investigated and to serve as a guide for possible future intensive studies. In such a preliminary survey it was practical to study each variable at two levels only, and hence the conclusions drawn from this investigation must necessarily be regarded as tentative since the effect of any variable is doubtless de-

pendent upon the range over which it is studied. Despite these limitations, certain aspects of the results obtained seem worthy of comment.

The results of the baking tests clearly indicate that the amylase activity of the malted wheat flours is the only property of any appreciable significance in their utility for diastating purposes. The most important aspect of the study is, therefore, a consideration of the relative significance of the various factors which affect amylase activity. Of the several variables studied, germination time was by far the most important, accounting for 82% of the total variance. In contrast, steeping level accounted for only 5.7%, wheat type 1.4%, and protein content 0.2% ; within the limits of the treatments applied, kilning was without any measurable effect. The remaining 11% of the total variance is ascribable to interactions and experimental error. As was previously indicated the relative magnitudes of these effects might be somewhat different had other levels been used in testing the respective factors. The malting conditions which produced the highest amylase activity—germination for five days and steeping to 44% moisture—also gave the lowest yield of malt. This loss, however, is of little consequence when compared with the increase in activity. Thus the mean activity for all wheats malted under the above conditions was five times that of the malts produced by steeping to 40% moisture and germinating for three days. The malting loss under the former conditions was 6.8% as against 2.0% for malts produced at the lower steep and shorter germination.

Since wheat type and protein level were without effect on malting loss, these variables may be considered entirely on the basis of their ability to produce malts of high amylase activity. Our studies show that hard red spring wheats give somewhat higher amylase values than durum; however, in no case was the difference large and the relative behavior of the two wheat types was not consistent at the two protein levels and for different malting treatments. This suggests that at different protein levels and under other malting conditions, the relative amylase activities of hard red spring and durum malt flours might well be different from those encountered in this study. The evaluation of wheat type might profitably be investigated over a range in protein content under fixed germination and optimum steeping conditions.

In regard to protease activity, the baking tests indicate that this property is not of appreciable importance with the samples studied under the specific malting conditions employed. It has previously been reported, for example by Sherwood and Bailey (1926a, 1926b), that increasing amounts of malted wheat had a deleterious effect on the baking quality of flours milled from blends with ungerminated material, particularly in the instance of wheats germinated for five days. These

authors suggest that the undesirable effects may have been caused by excessive proteolytic activity. Our findings are not in accord with this conclusion since our data indicate that when the proteolytic-activity values of the malts are compared on the basis of the quantities of malt required to produce a uniform gassing rate, the proteolytic activity of the five-day malts is actually only about one-half that contributed by the three-day malts. This method of estimating the relative proteolytic activity at constant gassing power conforms most closely to the commercial application of malted wheat flour for diastating purposes and therefore seems the most logical approach. It appears probable that the deleterious effects noted by Sherwood and Bailey were due to factors other than proteolytic activity. In view of the fact that these investigators report results based on the addition of amounts of germinated wheat of from 5% to 40% of the total, the harmful effects noted may have been due to excessive starch liquefaction.

It would indeed be surprising if the variations in proteolytic activity due to the addition of the various malt flours tested in the present study were found to be of appreciable significance. The proteolytic activity of the base flour to which the malts were added was 53 units per 100 g., as determined by the Ayre and Anderson technique (1939), while that contributed by the malt flours at the dosages employed varied from approximately 0.2 to 1.6 units; hence, the differences in the proteolytic activity thus contributed were negligible in relation to the total. Accordingly it may be concluded that, unless conditions widely different from those of this study are employed, and unless under these different conditions the ratio between amylase and protease activity is markedly changed, protease activity cannot be a factor of any material importance in the quality of malted wheat flours.

Summary

Representative composite samples of high-grade hard red spring and amber durum wheats, each at two protein levels differing by approximately 2%, were experimentally malted using eight different treatments representing combinations of steeping to 44% and 40% moisture content, germinating for five and three days, and kilning for 12 hours at 100° F. and for 12 hours at 100° to 130° F.

Growth and yield of malt were not affected by wheat type or protein content. Increasing the steeping level and germination time resulted in greater growth and lower yield; raising the kilning temperature decreased yield slightly.

Protein content of malted wheats and of flours experimentally milled therefrom decreased with conditions which tended to increase growth,

the effect being greater for flours. In general, decrease in ash was not significant.

Amylase activity, expressed as the reciprocal of the amount of malt required to produce constant gassing power and constant autolytic maltose production in blends with a common base flour, was influenced by all factors investigated except kilning. Increase in germination time and steeping level and the use of hard red spring wheat rather than durum raised amylase activity. The over-all mean difference between levels for these factors was 2.44, 0.64 and 0.31 units respectively. However, the difference between levels for any one factor was affected by variation in other factors. The highest activity was obtained with high-protein hard red spring wheat steeped to 44% moisture and germinated five days.

Autolytic protease activity of the malted wheat flour was increased with longer germination, higher steeping level, higher protein, and with durum wheat, the over-all mean difference between levels being 63.1, 60.5, 35.5 and 34.1 units respectively. The effect of steeping was conditioned by protein level and germination time; conversely the last two factors were influenced by steeping level.

Amylase and protease activity were significantly correlated ($r = +.676$). The ratio between these activities was markedly influenced by wheat type, protein content, steeping level, and germination time. At constant amylase activity, the lowest protease activity was obtained with low-protein hard red spring wheat steeped to 40% moisture and germinated five days.

Baking tests on blends of a common base flour with amounts of the various malt flours giving constant and normal gassing power revealed no significant difference in dough handling properties, loaf volume, and external and internal bread characteristics. Unless malting conditions exist which would produce a widely different ratio between amylase and protease activity than those encountered in this study, it would appear that protease activity is of little or no significance in evaluating wheat malts for diastating purposes.

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THE APPLICATION OF THE DROPPING MERCURY ELECTRODE TO THE STUDY OF OXIDATION-REDUCTION SYSTEMS IN FLOUR

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It was hoped that the polarograph would offer a valuable tool in the study of any possible oxidation-reduction systems present in flour. The polarographic method involves the determination of current-voltage curves in electrolysis experiments by means of the dropping mercury electrode. The polarograph makes possible a photographic record or polarogram of the current-voltage curve, which is obtained automatically by recording the deflection of a current-reading galvanometer in series with the electrolysis cell, across which a continually increasing e.m.f. is applied. For each reducible or oxidizable sub-

stance present in solution a characteristic "wave" is obtained on the polarogram.

Each wave consists of a region in which the current rises rapidly and levels off to a region of diffusion current, that is, a region of potentials over which the current is practically constant, and is determined by the rate of diffusion of reducible or oxidizable material to the surface of the dropping mercury electrode. In general, the diffusion current is proportional to the concentration of material undergoing electrode reaction. In a given electrolysis medium many oxidation-reduction systems are characterized by constant "half-wave potentials." Thus the potential at which the current has reached one-half of the diffusion current is determined by the nature of the electrode reaction and is independent of the concentration of diffusing material. For the theory of polarography and the interpretation of polarograms in general, reference should be made to the review papers of Kolthoff and Lingane (1939) on inorganic polarography and Müller (1939) on organic polarography.

Experimental

The electrolysis cell and dropping electrode were of the type described by Lingane and Laitinen (1939). A saturated calomel reference electrode (S.C.E.) was used in all experiments. Dissolved oxygen was removed by bubbling tank nitrogen through the electrolysis
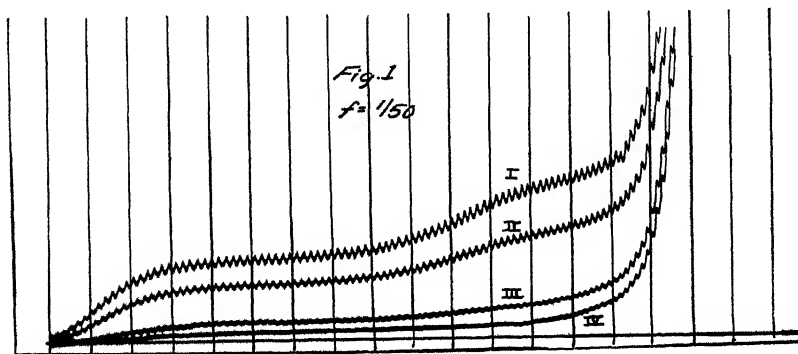
Fig. 1. Oxygen-reduction waves in presence of flour (Curve I) and effect of removal of oxygen by nitrogen (Curves II, III, and IV).

solution for 30 to 45 minutes. Oxygen gives current-voltage curves with two characteristic reduction waves (the first due to reduction to hydrogen peroxide, the second due to the reduction to hydroxyl ions). A series of oxygen-reduction curves is shown in Figure 1. Curve I illustrates a 10% suspension of a straight-grade flour saturated with

air.   Curves II, III, and IV illustrate the effect of bubbling nitrogen through the solution for 5, 15, and 35 minutes respectively.   The characteristic oxygen-reduction maximum on the curves has been completely eliminated by the presence of the flour.

The galvanometer sensitivity is reported as the fraction of the full sensitivity of 0.0205 microampere per mm. of deflection on the polarogram, and the currents as mm. of deflections at the particular sensitivity used.   All curves above the horizontal line represent a reduction (cathodic) process at the dropping mercury electrode while all curves below the horizontal line represent an oxidation (anodic) process at the dropping electrode.   In each polarogram, the current-voltage curves begin at an applied e.m.f. of 0.0.   Each abscissa division represents an increase of 0.1 volt in the negative potential of the dropping electrode.
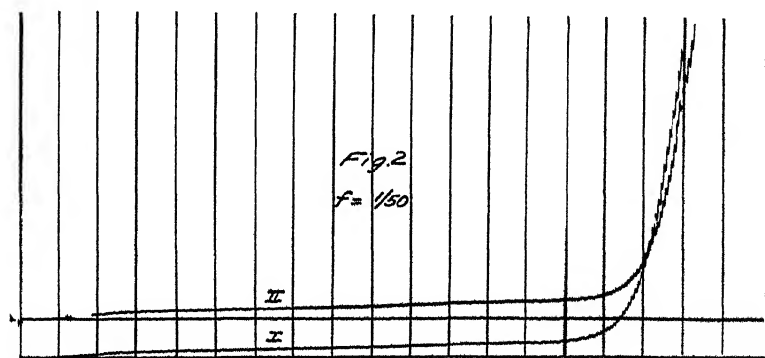
Fig. 2.   0.1N KCl extract of a spring-wheat straight.

The simplest extraction possible was first tried on an unbleached spring-wheat straight flour which had shown a strong bromate response. The flour was extracted with 0.1N potassium chloride for various lengths of time.   Six polarograms were run, of which a typical example is shown in Figure 2.   No characteristic waves were found over a range of potentials from + 0.2 to − 1.8 volt (S.C.E.).   Since no oxidation-reduction system was detected in the potassium chloride extract, it was thought that an extraction of the flour with an acetate buffer of pH 4.7 would perhaps be more successful.   Figure 3, Curve I, shows that no detectable amounts of electro-reducible or oxidizable substances were extracted.   A winter-wheat clear was used because it was thought that an unbleached clear would have more reducing substances.

. Next the unbleached straight flour was treated with 0.01% papain and then extracted with an acetate buffer.   A larger amount of papain
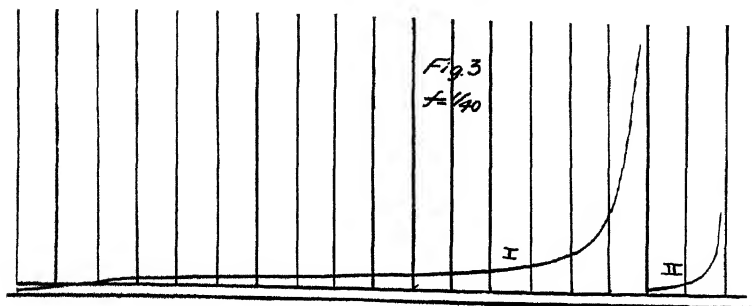
Fig. 3.   Acetate buffer extract of winter-wheat clear.

was also tried using for the extraction an acetate buffer of pH 4.7 containing 1% of suspended papain.   Both treatments failed to release oxidizable or reducible substances.

Since we knew that glutathione is present in wheat germ, a $0.1N$ potassium chloride extract of the germ was made.   A definite anodic wave is seen in Figure 4, Curve I.   In such an unbuffered solution, the
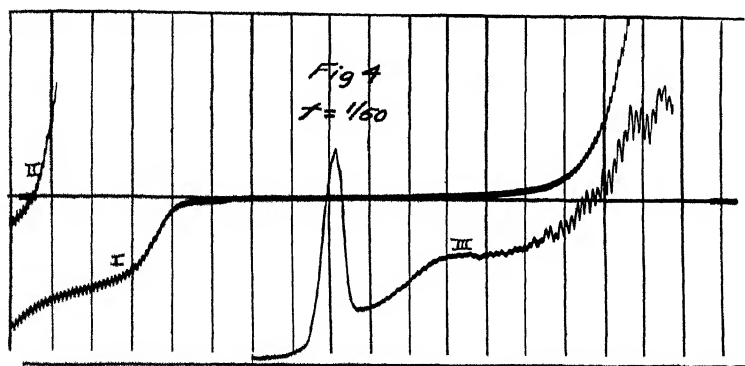
Fig. 4. $0.1N$ KCl extract of wheat germ.

half-wave potential does not have its usual significance.   Since hydrogen ions are involved in the electrode reaction the half-wave potential is not constant with changing glutathione concentration, because in unbuffered solution the pH of the solution at the electrode surface is a function of the current.   A decreasing pH shifts the anodic wave to more positive potentials.   When the germ was extracted in $1N$ hydrochloric acid, or upon acidifying the potassium chloride extract, the polarogram showed no anodic wave, since in a highly acid medium the wave is shifted to such positive potentials that it no longer appears.

Figure 5 shows that the anodic wave is due to glutathione extracted from the germ.   Curve I was obtained on an acetate buffer extraction
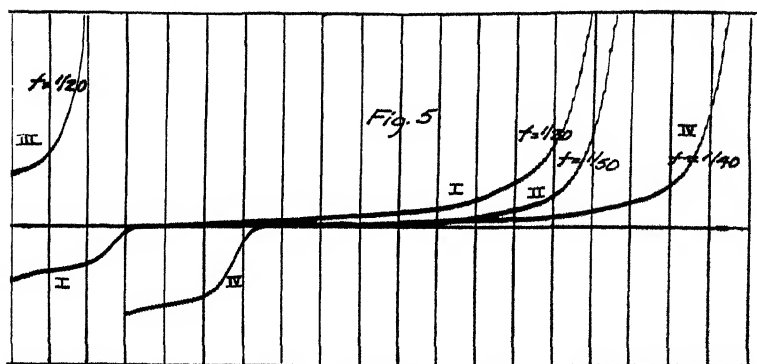
Fig. 5. Acetate buffer extract of wheat germ (Curve I) and after addition of glutathione (Curve II).

of wheat germ and Curve II was obtained after the addition of a small amount of glutathione solution.[1] A smooth curve occurring at the same potential was obtained, indicating that glutathione was responsible for the original wave. The effect of oxidation of the glutathione in wheat germ by treatment with Agene is shown in Figure 6, Curve I.
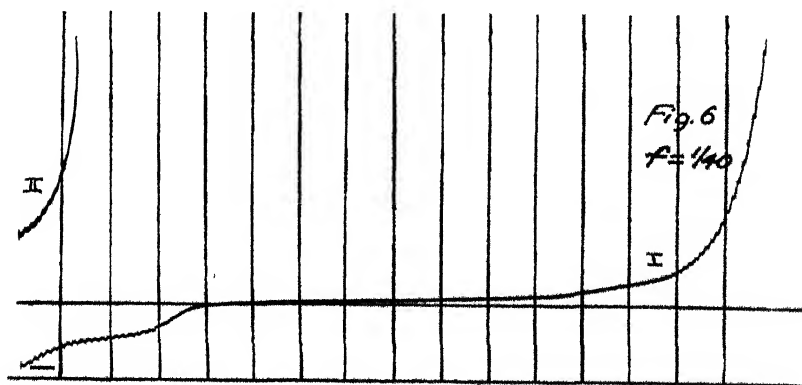
Fig. 6. Effect of oxidation of the glutathione of wheat germ by Agene.

The height of the wave was considerably decreased, but the wave did not entirely disappear. A heat treatment of the germ also decreased the height of the wave considerably.

Since germ but not flour gave an anodic wave using a potassium chloride extract, it was of interest to try the bran. A small amount of material, probably glutathione, was extracted from a winter-wheat bran using $1N$ potassium chloride as is shown in Figure 7, Curve II.

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[1] Curves III and IV, Figure 5; Curve II, Figure 6; Curves II–V, Figure 8; and Curves III and IV Figure 11 represent material not reported in this paper.
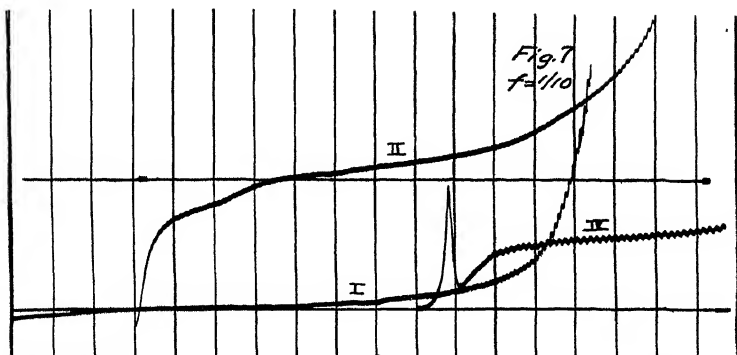
Fig. 7.   1N KCl extract of a hard winter wheat bran (Curve II).

The anodic wave disappeared in 1N hydrochloric acid medium as in the case of the germ extracts as a result of the high acidity of the medium.

Since we were sure that some reducing substance was present in the flour and since it could not be demonstrated by a simple extraction with an acetate buffer we made an acetate buffer extraction of two doughs (made with yeast, salt, sugar, flour, and water), one with potassium iodate and one without potassium iodate. No distinctive waves were obtained in either case.
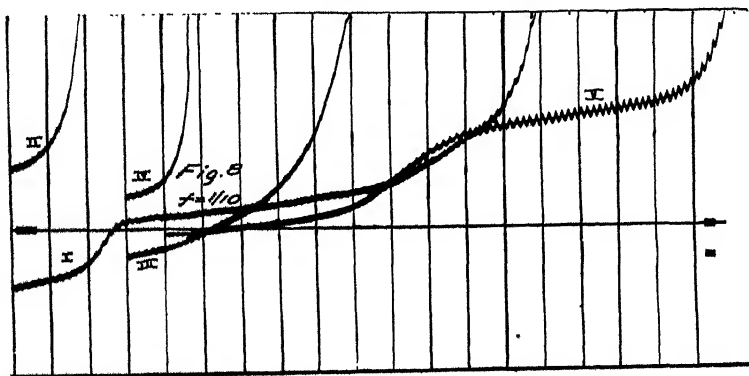
Fig. 8.   Acetate buffer extract of yeast (Curve I).

Figure 8, Curve I, however, shows the result of an acetate buffer extraction of yeast. A small anodic wave, probably due to glutathione, was obtained.

Since extractions with potassium chloride as well as with the acetate buffer failed to indicate the presence of a free reducing group in flour,

a more drastic extraction was tried.   Various grades and types of flour were extracted for definite times with $1N$ sodium hydroxide.   An excess of acetic acid sufficient to make an equimolar acetate buffer was added and the mixture centrifuged.   The supernatant liquid was used for the current-voltage curve.   An anodic wave with a half-wave potential of -0.15 volt (S.C.E.) was found.   The height of the wave increased with increasing extraction time.   After 24 hours of extraction no appreciable further increase in wave height was observed, showing a complete extraction after this period of time.   In all experiments reported a 24-hour extraction time was used.   Figure 9 shows a comparison of the anodic waves obtained by extracting 20 g.

Fig. 9.   Comparison of anodic waves of buffered alkaline extracts of patent (Curve III), clear (Curve I), and low-grade (Curve II).

of low-grade (Curve II), clear (Curve I), and patent (Curve III) flours from the same wheat mix, in 200 ml. of $1N$ sodium hydroxide, and buffering 50 ml. of the supernatant liquid with 50 ml. of $2N$ acetic acid.   The difference between these particular flours was not very striking, although the patent gave a noticeably smaller anodic wave.

It was shown that the anodic waves were caused by a reducing substance, since the addition of enough potassium iodate to make the solution 2×10^{-4} M caused the anodic wave to disappear almost completely.   The addition of iodate is discussed more in detail below.

In order to ascertain in what constituent of the flour the reducing substance was present a separation of the starch and gluten was made using a spring-wheat patent and a clear.   Two fractions of the starch obtained from both patent and clear by centrifuging were subjected to the alkaline extraction described above.   None of the starch fractions showed the presence of a substance giving an anodic wave.   On the other hand, alkaline extraction of the wet gluten of flour showed

an anodic wave greatly increased in height over that obtained from the same weight of the whole flour, indicating that all of the material responsible for the wave came from the gluten fraction. An ether-extracted flour gave practically the same anodic wave on treatment with $1N$ sodium hydroxide as the unextracted sample. Likewise the alcohol-ether extract of a dried gluten when treated with sodium hydroxide and buffered showed no reducing substance. Any S-H compound originally present in the fat would probably be oxidized to the S-S form, which is not detectable by a cathodic wave in the presence of substances strongly adsorbed on the mercury surface.

As is the case with the flour itself, the height of the wave was found to depend on the time of alkaline extraction of the gluten. This is evident from Table I, which shows the increasing diffusion current

TABLE I

EFFECT OF EXTRACTION TIME ON HEIGHT OF ANODIC WAVE

Time of extraction of gluten	Diffusion current
Hrs.	*Microamperes*
3.0	3.62
4.5	4.95
24.0	6.23

with increasing extraction time for the wet gluten of a spring-wheat clear.

A comparison of the glutens of patent, clear, and low-grade flours from the same wheat mix is given in Figure 10, Curves I, II, and III.
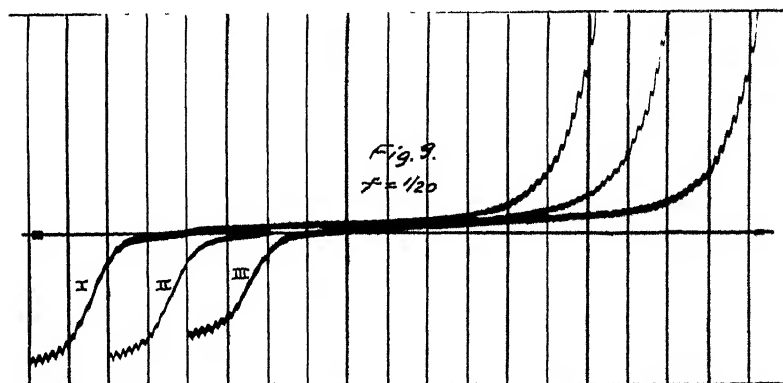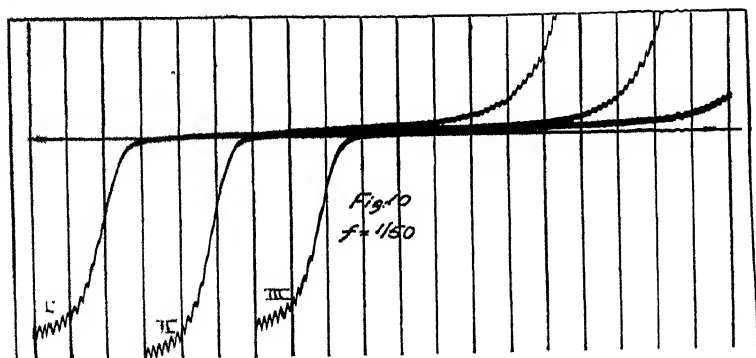

Fig. 10. Comparison of anodic waves of buffered alkaline extracts of gluten from patent (Curve III), clear (Curve II), and low-grade (Curve I).

These curves are based on glutens obtained from equal weights of flour. The results are summarized in Table II. It is to be noted that

TABLE II
Comparison of Glutens of Different Grades of Unbleached Flour

Flour (60 g.)	Wt. of wet gluten extracted	Diffusion current
	g.	Microamperes
Low-grade	25.8	5.82
Clear	27.2	6.43
Patent	22.0	5.61

on these particular flours the streams making up the clear and low-grade were such that the clear had a slightly higher protein and wet gluten content than the low-grade flour. The diffusion current is the higher the greater the amount of gluten.

Glutens from a spring-wheat clear flour which had been treated with Agene were compared with those from the unbleached flour. The results are given in Table III. Thus a slight decrease in wave

TABLE III
Effect of Agene on Height of Wave in Gluten Extract

Amount of Agene	Diffusion current
g.	Microamperes
none	6.23
10	6.02
20	5.71

height, but not nearly as large as one would expect, was obtained with Agene treatment.

Extractions of gluten were attempted using various dispersing agents in order to determine if the reducing substance could be released in any other way than by extraction with $1N$ sodium hydroxide. The solutions used were: 10% sodium salicylate, 10% urea, 30% urea, $2N$ acetic acid, $1N$ sulfuric acid, a borate buffer of pH 10, and a 3% yeast suspension. None of these reagents brought out a reducing substance giving an anodic wave. A complete hydrolysis to the amino acids by boiling for eight hours with 10% sulfuric acid, neutralizing and buffering, likewise failed to give a distinctive wave.

The effect of a treatment of flour with potassium iodate was of interest, since it was thought that the oxidized form of a reducing substance in the flour might give a distinctive cathodic wave on the current-voltage curve. Potassium iodate was chosen rather than potassium bromate since on the dropping mercury electrode, bromate is reduced at much more negative potentials than iodate, and it was desired to determine whether any of the oxidizing agent was reduced

after various times of standing from the height of its polarographic wave.

Figure 11 shows the waves obtained with the equal concentrations of potassium iodate in an equimolar acetate buffer in the absence (Curve I) and the presence (Curve II) of a 10% suspension of a spring-wheat clear flour after centrifuging. In the presence of the flour, the iodate wave was shifted to much more negative potentials, and the diffusion current was considerably decreased. The wave obtained in the presence of flour was shown to be caused by iodate, since the addition of more iodate simply caused the wave height to increase proportionally. The decrease in wave height did not appear to be caused by the reduction of part of the iodate, since no reduction products of iodate appeared. Thus iodine was found to give a cathodic wave beginning at − 0.1 volt (S.C.E.) in the presence of flour, and iodide
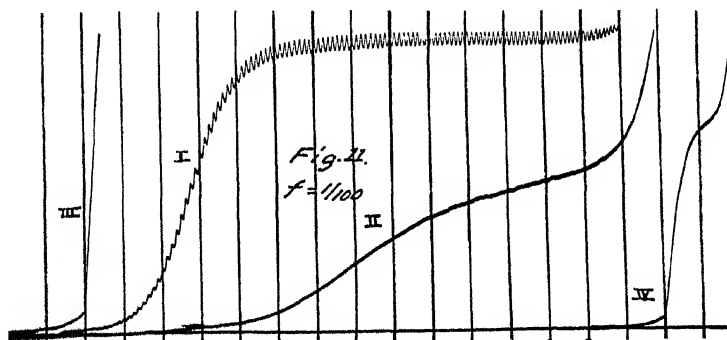
Fig. 11.   Potassium iodate curves in acetate buffer in absence (Curve I) and presence (Curve II) of a centrifuged flour suspension.

gives a characteristic anodic wave beginning at about the same potential. Furthermore, the time of standing did not affect the height of the iodate, indicating that no slow reactions occurred.

In order to determine whether the decreased height of the iodate wave in the presence of flour was caused by a simple viscosity effect of the medium, the diffusion current of a metal ion was measured in the presence and absence of a flour suspension. Thallous ions were chosen because of their low tendency to form complexes. Figure 12 shows the polarographic waves of 10^{-3} M thallous chloride in an equimolar acetate buffer in the absence (Curve I) and presence (Curve II) of a 10% centrifuged flour suspension. The diffusion current was changed less than 1% and the half-wave potential was identically the same in the two cases. It may be mentioned that a 2% change in viscosity would be necessary to make a 1% change in the diffusion current, since the diffusion current varies as the square root of the

Positive tests for mercury were obtained after electrolyzing the following solution: cysteine in an acetate buffer, extract of wheat germ in an acetate buffer, and the buffered solution containing an alkaline extract of gluten.

In the case of cysteine and glutathione, the positive mercury tests indicate that the anodic waves are caused by the formation of mercury complexes with the -SH groups of the type RSHg. The reducing substance released from gluten by sodium hydroxide also forms a stable mercury complex, an observation which might indicate a sulfur compound was responsible.

Our previous work (Sullivan, Howe, Schmalz, and Astleford, 1940) has indicated quite definitely that some grouping present in the gluten of flour is responsible for the action of certain improvers whether they are oxidizing agents or reducing substances causing an oxidation-reduction system. The theory of Jørgensen and of Balls and Hale that the inhibition of the proteolytic enzymes explains the beneficial effect of substances such as bromate or nitrogen trichloride does not seem tenable to us.

Doubtless the proteases of flour are of the papain type and are activated and inhibited by the same reagents as papain but we feel that the action of the proteolytic enzymes present in sound normal flour is of a great deal less importance than is that of the reducing groups liberated by the gluten itself during fermentation.

Unfortunately, in the present study, we were unable to release these reducing groups by any means other than normal sodium hydroxide. It was difficult also to do much with a fermented dough. The limitations of the mercury electrode are such that it is difficult to prove, in the case of dough, the presence of a reducing substance during fermentation since complex formation of certain substances occurs with the flour or dough.

Recent experiments have shown that treatment of the sodium hydroxide extract of gluten with hydrogen sulfide, buffering and removing the hydrogen sulfide with nitrogen or hydrogen greatly increased the height of the anodic wave, indicating the liberation of a greater amount of reducing substance. With this hydrogen sulfide treatment it was found that approximately one quarter of the reducing substance came from the lipids and about three quarters from the remainder of the gluten.

Conclusion

A substance giving an anodic wave (indicating a reducing substance) was released from the gluten on treatment with sodium hydroxide but not from the starch. A variety of other treatments failed to liberate

the substance. A buffered solution containing the alkaline-treated wet gluten gave a positive test for mercury on electrolysis, indicating that there was formation of a mercury complex with the material in question. Certain RSH compounds give similar mercury complexes. The exact nature of the group giving that anodic wave is not known but indications are that it may be some reduced sulfur linkage.

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SELENIUM IN WHEAT AND WHEAT PRODUCTS

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For many years there has been known a livestock disorder which occurs in parts of the semiarid Great Plains of the United States. This disorder is known in the plains as "alkali disease," a misnomer applied by early settlers who attributed the trouble to alkali (high salt) water. The disorder is now known to be selenium poisoning and is caused by the ingestion of vegetation that has absorbed this toxic element from the soil (Beath, Draize, and Gilbert, 1934; Byers, 1936; Franke, Rice, Johnson, and Schoening, 1934). It has been found that relatively small amounts of selenium taken daily disturb the physiological processes. Rats on an adequate diet prepared to contain 6 ppm. (parts per million) of selenium, by the incorporation of seleniferous cereals, were found to be considerably below normal in weight. Furthermore the number of young born and the percentage reared were less than normal. A similar diet containing 3 ppm. of selenium had a slight effect on reproduction, although growth was apparently normal (Munsell, De Vaney, and Kennedy, 1936). During field studies in affected areas we have observed selenized animals suffering

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various gradations of affliction. Pigs have been encountered that were nearly hairless from ingestion of toxic grain, horses without tail or mane, and with deformed hoofs, cows with poor coats and loose hoofs, and bulls impotent from seleniferous forage. These symptoms are accompanied by poor general condition of the affected animals. Farms were encountered where it was necessary to purchase eggs for setting, since the eggs from the flock would not hatch.

Many farmers are familiar with seleniferous "alkalied" grain, and use various methods to circumvent its toxicity. Some farmers sell their grain and buy good grain for feeding. Others feed a mixture of seleniferous grain with good grain from other fields on their own farm or obtained elsewhere.

Because of numerous inquiries concerning the health hazards incurred by people outside of seleniferous areas, it seemed of importance to determine to what extent foodstuffs which may come into the markets are likely to contain significant amounts of selenium. Therefore a comprehensive survey of the grain crops of areas known to be seleniferous was made by one of the authors during the harvest season of 1937. This survey was supplemented by samples furnished by the U. S. Food and Drug Administration and the General Mills Company, Inc. In this manner, 950 samples of wheat and wheat products were obtained, together with about 300 samples of barley, corn, oats, and rye. These samples were analyzed and the results will be given in detail in a technical bulletin of the U. S. Department of Agriculture (Williams, Lakin, and Byers, 1940). It is considered advisable by the authors to make immediately available a résumé of the results obtained from the determination of selenium in the samples of wheat and wheat products.

Wheat and wheat products were collected in portions of Colorado, Kansas, Nebraska, South Dakota, Wyoming, Montana, North Dakota, and Minnesota. The places of collection were in the seleniferous areas or, if outside the areas, in those towns that might handle some grain from such areas. In Table I the counties in which samples were collected are listed for each state involved in the survey except Minnesota. The six samples obtained from this state were obtained at grain centers believed to handle grain from seleniferous areas farther west. Areas of soils capable of producing seleniferous grain are small and are interspersed with soils of much lower selenium content. The percentage of the land in the counties listed in Table I, known or thought to be seleniferous, varies over a wide range, but in no case could it be said that all of the soils in any of these counties are seleniferous.

TABLE I

DISTRIBUTION OF POINTS OF COLLECTION BY COUNTIES WITHIN THE STATES

State	Counties		
Colorado.	Bent	Otero	Rapahoe
	Crowley	Prowers	
Kansas	Gove	Logan	Rooks
	Graham	Ness	Trego
	Lane	Phillips	Wallace
Montana	Big Horn	Glacier	Roosevelt
	Blaine	Hill	Sheridan
	Carter	Judith Basin	Teton
	Cascade	Liberty	Toole
	Choteau	Petroleum	Valley
	Daniels	Phillips	Yellowstone
	Fergus	Pondera	
Nebraska	Boyd	Harlan	Red Willow
	Dawes	Hitchcock	Sheridan
	Furnas	Knox	Sioux
North Dakota	Burleigh	Ward	Williams
	Mountrail		
South Dakota	Brule	Haakon	Meade
	Butte	Hughes	Pennington
	Custer	Jackson	Stanley
	Fall River	Jones	Tripp
	Gregory	Lyman	
Wyoming	Albany	Crook	Niobrara
	Big Horn	Goshen	Platte
	Campell	Johnson	Sheridan
	Converse	Natrona	Washakie
			Weston

Samples of wheat were collected primarily at elevators in shipping centers. Wheat and wheat products were obtained from flour mills. Also, samples were collected from fields, storage bins on farms, and from trucks hauling from farms. It naturally follows that the individual samples are representative of various sources of wheat ranging in quantity from a few bushels to many thousand.

Discussion of Data

Of the 951 samples examined, 82.5% contained 1 ppm. or less, 10% contained 2 to 3 ppm., and 7.5% contained 4 ppm. or more of selenium. Only 8 samples contained as much as 10 ppm. or more of selenium. A more detailed analysis of the data by states is summarized in Table II.

Since there is little in the literature which permits an appraisal of the selenium intake tolerated by human beings, without injury, all references to toxicity in this discussion relate to experimental or domestic animals. Certainly those samples that contain 4 ppm. or

TABLE II

Distribution of 951 Wheat and Wheat Product Samples from Seleniferous Areas in Eight States, Arranged on the Basis of Selenium Content

Location	Total number of samples	Percentage distribution on basis of selenium content						
		0.1 ppm. or less	0.2 ppm.	0.5 ppm.	1 ppm.	2 ppm.	3 ppm.	4 ppm. or more
		%	%	%	%	%	%	%
Colorado	16	6	6	25	44	6	13	0
Kansas	61	1.5	6.5	36	26	20	5	5
Montana	410	15.9	25.6	35.4	19.5	3.2	0.2	0.2
Nebraska	106	13.2	16.0	21.7	19.8	8.5	8.5	12.3
North Dakota	84	3.6	29.8	39.3	22.6	3.6	1.1	0.0
South Dakota	188	2.1	11.2	14.5	15.4	15.9	10.6	30.3
Wyoming	80	18.7	31.2	40.0	8.8	0.0	1.2	0.0
Minnesota	6	0.0	67	33	0.0	0.0	0.0	0.0

more, if they constitute the whole diet, would be toxic to white rats. From this viewpoint the vast majority of the samples examined cannot be considered toxic. Only those samples collected in seleniferous areas in Nebraska and South Dakota show an appreciable percentage containing 4 ppm. or more. Many of these samples are from farms and represent relatively small quantities of wheat.

In two adjacent towns in a particularly toxic area in South Dakota, 25 samples of wheat were collected from elevator bins and freight cars. Twenty of these samples contained 4 to 8 ppm. of selenium. These samples would be toxic to white rats, and the 14 samples containing in excess of 5 ppm. would adversely affect the hatchability of chicken eggs if used as the total laying ration (Poley and Moxon, 1938).

Flour was collected at mills in South Dakota, Nebraska, Wyoming, Montana, and North Dakota. Of the 66 samples of flour examined, only 5 contained in excess of 1 ppm. of selenium. Two samples of flour from a small roller mill in South Dakota contained 4 and 5 ppm. of selenium. The wheat, bran, middlings, and wheat cleanings collected at this mill also had a selenium content of 4 to 5 ppm. A similar situation was observed in northwestern Nebraska. From a small mill in this area a sample of flour was found to contain 4 ppm., bran 4 ppm., shorts 6 ppm., and wheat 4, 10, and 0.5 ppm. of selenium. One should not infer from these data that these two mills always produce flour containing 4 to 5 ppm. of selenium. These samples merely represent the flour sampled at the time the mills were visited. Other mills in seleniferous areas may occasionally produce flour of higher selenium content than was found in their flour at the time they were visited. The data show that a very small percentage of the flour produced in seleniferous areas contains selenium in excess of the minimum requirement for injury to white rats.

The data used in Table II and discussed above represent wheat and wheat products in, or adjacent to, seleniferous areas. The wheat from these areas constitutes, in all cases with the possible exception of Montana, a minor portion of the wheat production of the states involved. Therefore it is to be emphasized that the percentage distribution shown in the table represents only the wheat produced from portions of the states in which selenium is known to be a possible component in significant quantities. Furthermore, these samples are representative of the grain produced in the United States most likely to be toxic due to selenium, and only 7.5% of the samples contain sufficient selenium to be toxic to white rats when used as the *whole* diet. Since the wheat production of seleniferous areas in the United States is a minor portion of the total crop, one may reasonably presume that the seleniferous wheat is greatly diluted when shipped to large milling centers and that only in mills located in seleniferous areas could one expect to find flour containing significant amounts of selenium.

Selenium has been found in all samples of wheat examined in this laboratory, when sufficient material has been used in the analysis. Robinson (1936) has examined wheat from Argentina, Australia, Canada, Hungary, Mexico, South Africa, and Spain, in addition to domestic samples. He has found selenium in all of the samples examined. The authors agree with Robinson that it is improbable that any field-grown wheat is entirely free from selenium, since, when carefully examined, wheat has always been found to contain selenium. The mere presence of selenium in wheat must not be viewed with alarm since it is probably a normal constituent and becomes a problem only when it occurs in sufficient quantities to be injurious.

Summary

The selenium content of 951 samples of wheat and wheat products from seleniferous areas in the United States was determined and a résumé of the results given. The vast majority of these samples were low in selenium content. Samples of wheat and wheat products containing sufficient selenium to be toxic to domestic animals were found. From the known facts the authors believe selenium to be present in all wheat in detectable amounts.

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DOUGH OXIDATION AND MIXING STUDIES. IV. EFFECTS OF OXYGEN AND POTASSIUM BROMATE IN SPONGE DOUGHS

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It has previously been reported (Freilich and Frey, 1939) that bromate in straight doughs was found to produce effects, in addition to the inhibition of proteases, which were partly dependent on dough fermentation products. Remixing straight doughs after fermentation overcame certain " excess bromate " effects. Oxygen was found to inhibit protease activity and diminish the harmful effects of wheat germ.

Experimental

In the present study 60%–40% sponges were used with 1.5% yeast in the sponge and 1.67% salt, 5% sugar, and 3% shortening in the dough, with variable absorption. Sponge time was generally $3\frac{1}{2}$ hours at 86° F., dough time 20 minutes, bench proof 15 minutes, pan proof to constant height at 100° F., and oven temperature 410° F. All doughs were scaled to 480 g. and molded by machine. Mixing was done in a specially adapted Hobart-Swanson mixer.

Tests were made to determine the effects of bromate and oxygen on doughs fortified with protease (papain) and wheat germ. Unless otherwise stated these latter ingredients were used at levels of 0.014% and 3% in the sponge and 0.0093% and 2% in the dough, respectively.

Figure 1 shows the action of bromate in counteracting the effects of the added protease. Normal loaves were obtained with bromate dosages of 25–50 mg. per loaf, but a slight excess bromate effect appears at 100 mg. Dough time is an important factor in the appearance of
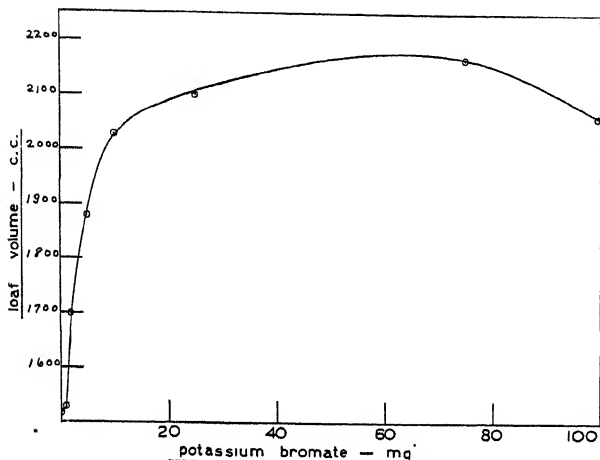
Fig. 1.   Effects of potassium bromate on loaf volume of sponge doughs with added papain.

this phenomenon; using 20, 90, and 105 minutes of dough time, loaf volumes of 2,220, 2,060, and 2,020 cc. were obtained with increasing excess-bromate effects in the last two (see Fig. 2).   It is to be noted that
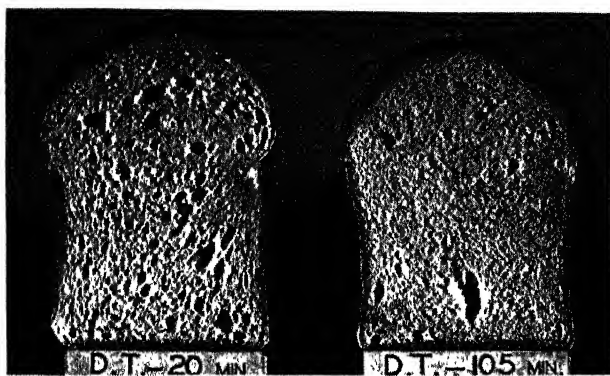
Fig. 2.   The " excess bromate " effect in sponge doughs, due to longer dough time (D.T.) after remixing.

the excess-bromate effects here appeared after remixing of the sponges. Since this effect was eliminated in straight doughs by remixing, it is evident that we are dealing with a reversible type of reaction:

$$\begin{array}{ccc} \text{normal} & \xrightarrow{\text{bromate + fermentation}} & \text{excess bromate} \\ \text{properties} & \xleftarrow[\text{remixing}]{} & \text{effects} \end{array}$$
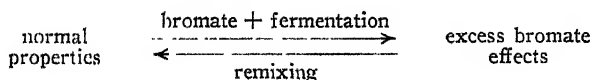
Table I and Figures 3 and 4 indicate the similar effect of oxygen, except that an " excess " effect is not obtained.   We have not yet ob-

TABLE I

EFFECT OF OXYGEN IN SPONGE DOUGHS

	Loaf volume		
Sponge mixing time	Sponge, N₂ Dough, air	Sponge, O₂ Dough, air	Sponge, O₂ Dough, O₂
min.	*cc.*	*cc.*	*cc.*
1	1610	1660	1650
2	1550	1690	1720
3	1580	1770	1870
4	1560	1900	1960
5	1560	1940	1960
10	——	1970	1990
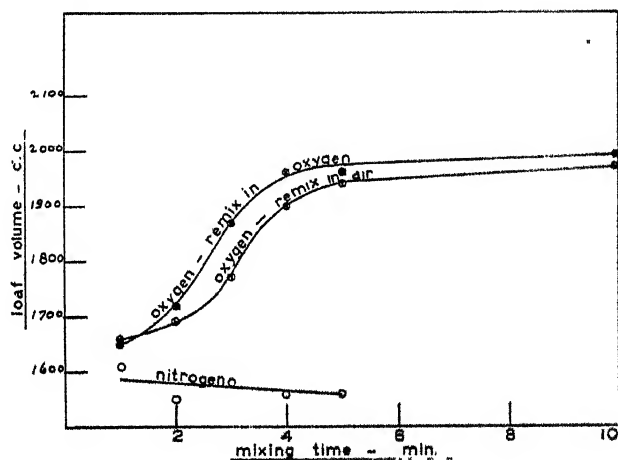
Fig. 3. Effects of mixing in oxygen and nitrogen on loaf volume of sponge doughs with added papain.
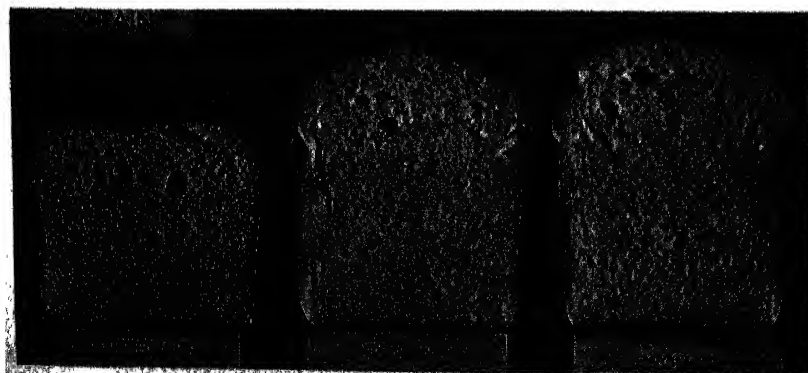
Fig. 4. Sponge doughs with added papain mixed in nitrogen, oxygen, and with added bromate.

tained excess-bromate effects with oxygen even with excessive amounts. Indeed, remixing in oxygen still produces improvement in these cases, indicating that the use of oxygen in the sponge only may not be sufficient for optimum results.

Three samples of wheat germ were tested with the results shown in Table II and Figure 5. Grinding sample C increased its deleterious effect, but oxidation successfully overcame it.

TABLE II

WHEAT GERM

Mixing times: sponge 4 minutes, dough 3 minutes.

Wheat germ	Loaf volume	
	Nitrogen	Oxygen
	cc.	*cc.*
A	2000	2140
B	2050	2150
C	1980	2140
C (ground)	1860	2210
C (ground)	2180[1]	—

[1] 10 mg. KBrO₃ in the sponge.
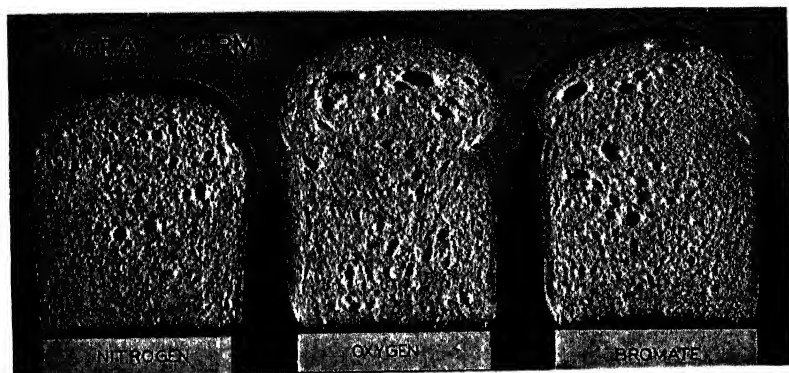
Fig. 5. Sponge doughs with added wheat germ mixed in nitrogen, oxygen, and with added bromate

That flours differ in their response to oxygen is indicated by the results given in Table III and Figures 6 to 10. In these experiments no added protease or wheat germ was used. Flours No. 7 and No. 8 show slight deleterious effects with oxygen, while this negative response is still more pronounced with flour No. 4. Thus the oxygen requirements of flours may vary over a considerable range. Pan proof times with flours No. 7 to No. 11 and 12 to 14 decreased with a lowering of

TABLE III
Effect of Oxygen on Various Flours

No.	Flour	Sponge mixing time	Loaf volume	
			Nitrogen	Oxygen
		min.	*cc.*	*cc.*
1	Southwestern straight	1	1950	1960
	Southwestern straight	5	2000	2160
2	Kansas Standard Patent	1	1910	2020
	Kansas Standard Patent	2	1960	2070
3	Kansas, No. 1	–	1940	2080
4	Kansas, No. 2	–	2070	1890
5	Straight grade, unbleached	–	2000	2160
6	Straight grade, bleached	–	2080	2160
	Northwestern Wheat:			
7	Short Patent	3	2130	2120
8	Long Patent (bleached)	3	2200	2140
9	Straight (bleached)	3	2150	2230
10	Clear	3	2140	2210
11	Low grade	3	1690	2010
	Texas Wheat:			
12	Patent	3	1960	1980
13	Clear	3	1920	2050
14	Low grade	3	1760	2070
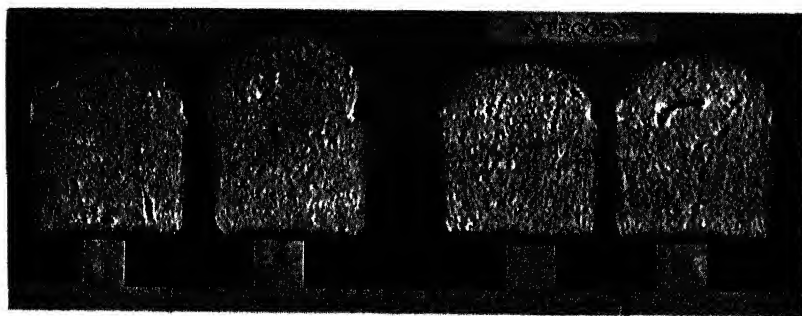
Fig. 6. Effects of mixing in oxygen and nitrogen on sponge doughs made from a Southwestern straight flour.

Fig. 7. Effects of mixing in oxygen and nitrogen on sponge doughs with two different Kansas flours.

Fig 8.   Effects of mixing in nitrogen and oxygen on sponge doughs made from the unbleached and bleached lots of the same flour

Fig. 9.   Sponge doughs made from the different grades of flour that were milled from the same Northwestern wheat, mixed in nitrogen and oxygen.

Fig. 10.   Sponge doughs made from the patent, clear, and low grade flours that were milled from the same Texas wheat, mixed in nitrogen and oxygen.
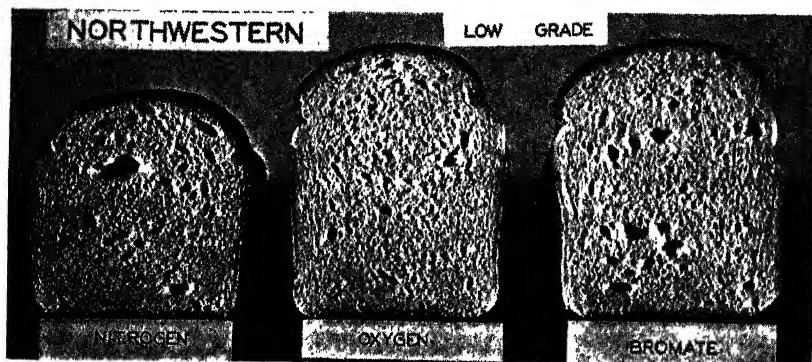
Fig. 11. Sponge doughs with bromate compared to mixing in oxygen and nitrogen, made from Northwestern low grade flour.
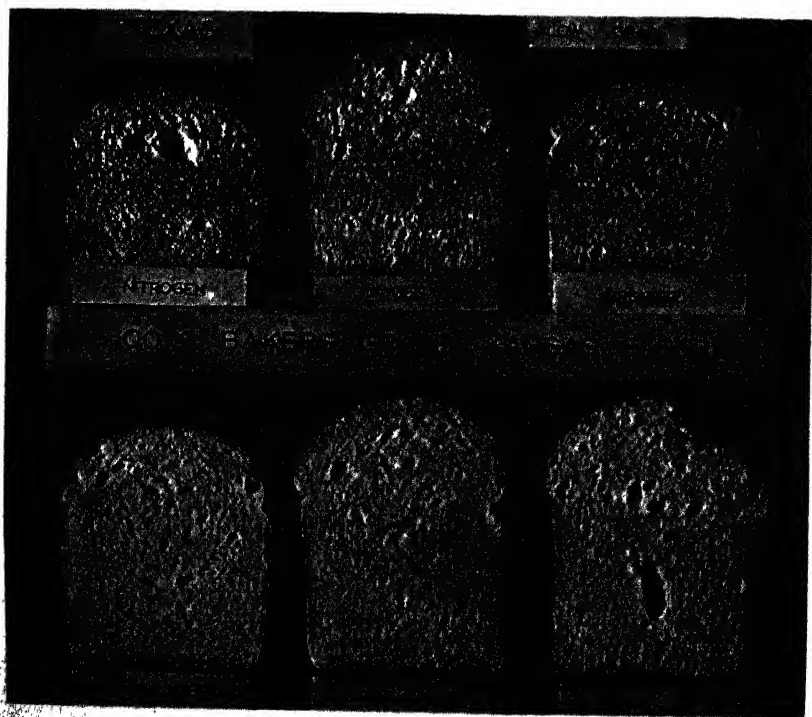
Fig. 12. Sponge doughs with bromate compared to mixing in oxygen and nitrogen, made from Texas low grade and 100% Kansas flours.

the flour grade, an effect particularly pronounced in the case of nitrogen, indicating an increase in soluble nitrogen constituents. These were probably produced partly as a result of increased proteolytic degradation.

Flours also differ in their relative responses to oxygen and bromate, as indicated in Table IV and Figures 11 and 12.

TABLE IV

Effects of Oxygen and Bromate (10 mg./loaf) with Certain Flour Types

Flour	Loaf volume		
	Nitrogen	Oxygen	Bromate
	cc.	cc.	cc.
Northwestern, low grade	1690	2010	2010
Texas, low grade	1760	2070	·1800
100% bakers' grade, Kansas	2010	2130	2220

The Texas low grade responds much better to oxygen than to bromate, while the reverse is true with the 100% bakers' grade Kansas flour. These varying results with oxygen and bromate suggest the possibility that their effects in flour may be different, though the final results of their action are similar in many respects.

Discussion

The effects of bromate and of oxygen in sponge doughs with added papain, wheat germ, and with different flours, are found to be generally similar to their effects in straight doughs. There is a difference, however, with respect to the excess-bromate effect. In conventional straight doughs, large amounts of bromate will always produce this effect, but in conventional sponges it will ordinarily not occur, until relatively enormous amounts of bromate are used, because of the remixing after fermentation and the short fermentation time used following the remixing.

There is a difference between ordinary sponges and those mixed in oxygen with respect to mixing time. Ordinarily, the sponge-mixing time may be varied over a wide range without affecting the quality of the resulting bread (Frey, Freilich, and Ekstedt, 1937); for this reason bakers use a very short sponge-mixing period, in comparison to the time of remixing. But when using oxygen alone, a longer mixing time is advantageous in order to incorporate sufficient oxygen for best results. With bromate, a normal sponge-mixing time may be used because it is usually effective even in the absence of oxygen.

The fact that flours differ in their oxygen requirements emphasizes the importance of proper control of the oxidation treatment which they receive.

The difference between the effects of bromate and oxygen with certain flours furnishes some evidence in support of the idea that there may be some fundamental differences between their actions in dough.

Summary

Bromate and O_2 incorporated by mixing counteracted the effects of added protease and wheat germ and improved the bread made from lower-grade flours by the sponge process. " Excess bromate " effects appear only if dough time is greatly prolonged after remixing. Flours vary considerably in their oxidation requirements. Some flours respond better to O_2 than to bromate, while with others the situation is reversed.

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A SIMPLE PHOTOMETRIC METHOD FOR DETERMINING THE PROTEIN CONTENT OF WHEAT FLOUR

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The Kjeldahl method, or one of its various modifications, is used extensively for the routine determination of protein in wheat and wheat flour. The method is reliable, fairly rapid, and is well adapted to routine work. Certain shortcomings of the Kjeldahl method, however, definitely limit its usefulness. In order to handle a large volume of work, a protein laboratory must be equipped with elaborate and very expensive equipment which must be permanently installed and is, therefore, nonportable. Large quantities of strong acid and alkali must be used, and noxious fumes and excessive heat are characteristic of many protein laboratories.

The Agricultural Marketing Service is interested in the development of a simplified procedure by which protein determinations may

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be made conveniently in small laboratories without the installation of cumbersome equipment. Such a method may be of considerable value in certain types of grain-inspection work.

The method herein described has been developed by utilizing certain physico-chemical properties of the gluten proteins, and is not based on any previously established procedure. It should be mentioned, however, that several colorimetric and turbidimetric methods for determining the protein content of various body fluids have been developed in recent years and are being used to advantage in clinical laboratories for the routine analysis of blood serum, urine, and cerebro-spinal fluid. Since the photometer is used with success in some of these clinical methods, it was believed that a photometric method might be developed for the determination of protein in cereal products.

The proteins of wheat flour are readily peptized by very dilute alkali. When an alkaline solution containing the peptized flour proteins is neutralized, the gluten proteins are precipitated. If, however, the protein sol is sufficiently dilute, and its hydrogen-ion concentration after neutralization is controlled by a suitable buffer, the gluten proteins will not separate from the solution but will nevertheless appear as a turbid and stable colloidal suspension. The degree of turbidity of such a suspension is a measure of the gluten protein content of the flour.

Experimental

To determine the optimum hydrogen-ion concentration and the minimum time required for the production of the greatest turbidity in a neutralized alkaline extract of wheat flour, 5-ml. portions of a filtered $0.05N$ potassium hydroxide extract of a flour were added to 25-ml. portions of a series of buffer solutions. The buffers covered a range in pH values from 6.0 to 8.7, and were prepared by mixing $M/5$ solutions of Na_2HPO_4 and KH_2PO_4 in various proportions. The relative degrees of turbidity of the suspensions produced were determined by measuring the light transmission through a definite depth of suspension by means of a photoelectric photometer, using a light filter with a maximum light transmission at a wave length of 530 millimicrons. The values obtained are given in Table I.

It is evident that under the conditions used the maximum turbidity (minimum light transmission) is attained by using a buffer of pH 7.8, which results in a suspension having a pH of approximately 8.2. It is also evident that the maximum turbidity occurs in about 60 minutes after the addition of the buffer to the extract. To obtain maximum turbidity, however, this length of time does not need to be exact, since the change in turbidity between 45 and 90 minutes is very small. It

TABLE I

TRANSMISSION OF LIGHT AT ±530 mμ. THROUGH SUSPENSIONS PREPARED BY ADDING
5-ML. PORTIONS OF A FILTERED 0.05N KOH EXTRACT OF A WHEAT FLOUR
TO 25-ML. PORTIONS OF M/5 PHOSPHATE BUFFERS

Suspensions contained in matched 7 × 7/8″ test tubes.

Suspen-sion No.	Buffer formula		H-ion concentration		Light transmission after				
	M/5 Na₂HPO₄	M/5 KH₂PO₄	Buffer	Sus-pension	15 min.	45 min.	60 min.	90 min.	24 hrs.
	Volumes	*Volumes*	*pH*	*pH*	%	%	%	%	%
1	100	0	8.72	9.63	62.3	60.4	59.9	59.6	63.4
2	99	1	8.39	9.47	57.7	55.6	55.3	55.2	56.7
3	97.5	2.5	8.10	9.10	55.1	53.5	53.2	53.2	53.7
4	95	5	7.86	8.30	52.9	52.0	51.7	51.7	52.8
5	94	6	7.80	8.16	52.3	51.5	51.2	51.3	51.9
6	93	7	7.71	8.02	52.8	51.9	51.6	51.8	52.5
7	92	8	7.63	7.83	53.2	52.0	52.0	52.2	52.8
8	91	9	7.60	7.76	53.5	52.5	52.2	52.3	53.2
9	90	10	7.51	7.62	54.5	53.1	52.7	52.7	53.2
10	80	20	7.23	7.31	55.5	54.3	53.9	53.9	55.3
11	70	30	6.98	7.08	56.7	55.1	55.1	55.1	56.1
12	60	40	6.79	6.88	57.5	56.6	56.5	56.3	57.7
13	50	50	6.61	6.72	60.1	59.4	59.0	58.9	60.6
14	40	60	6.43	6.50	62.7	62.2	61.9	61.8	65.6
15	30	70	6.26	6.36	66.9	66.6	66.4	66.3	86.4 [1]
16	20	80	6.03	6.18	68.9	68.5	68.2	68.2	92.3 [1]

[1] Precipitate settled out.

was further shown that the temperature of the suspensions had very
little effect on the final degree of turbidity, at least within the ordinary
range of laboratory temperature. For very exact work, however, it is
possible that the temperature should be controlled or that a tempera-
ture correction factor should be used.

The following method, based on the foregoing experiments, was
used to determine the relationship between the turbidity produced
and the protein content of the flour:

(1) To exactly 0.5 g. of flour in a 200-ml. Erlenmeyer flask,
add exactly 5 ml. of 95% ethyl alcohol (to prevent the flour from
coalescing) and 100 ml. of 0.05N KOH.

(2) Shake mixture intermittently for 15 minutes and then
centrifuge for 10 minutes at approximately 1600 rpm.

(3) To exactly 5 ml. of the centrifugate in a photometer test
tube (one of the selected tubes for use in lieu of an absorption cell),
add exactly 25 ml. of a buffer solution made by mixing 6 parts by
volume of M/5 KH₂PO₄ with 94 parts by volume of M/5 Na₂HPO₄.
This buffer solution should have a pH of 7.8. Mix the contents
of the test tube by inversion and allow to stand for 1 hour.

(4) Determine the transmission of light through the solution
in the test tube with a photoelectric photometer, using a light
filter having a maximum transmission at a wave length of 530
millimicrons. (Other wave lengths will give different but equally
satisfactory results.)

Thirty-four samples of flour, representing all classes of domestic wheat except durum, were analyzed by this method. These flours ranged in protein content from 7.30% to 16.26%, and in ash content from 0.38% to 0.61%. The results are given in Table II and are shown graphically in comparison with the protein content values as determined by the conventional Kjeldahl method in Figure 1.

TABLE II

SHOWING THE RELATIONSHIP BETWEEN PROTEIN CONTENT AND LIGHT TRANSMISSION
VALUES ON A SERIES OF 34 FLOUR SAMPLES

Sample No.	Class and variety of wheat from which flour was milled		Protein content ("as is" basis)		Light transmission at ±530 mμ (in 7 × 7/8" test tubes)
			Total	Not peptized by 5% K_2SO_4	
			%	%	%
1	White (Other than Federation or Baart)		7.30	5.85	70.4
2	White Club		7.66	6.18	69.0
28	White		8.61	6.89	62.8
29	White		8.77	6.98	62.1
3	White Federation		8.80	6.86	65.4
32	Soft Red Winter	Purkoff	9.13	7.39	58.7
34	Soft Red Winter	Nittany	9.31	7.63	57.6
33	Soft Red Winter	Red Rock	9.44	7.59	58.3
4	Hard Red Winter		9.44	7.91	55.9
5	White (Other than Baart)		9.70	8.16	56.7
26	White		9.79	7.96	56.4
6	White Baart		10.09	8.46	55.0
19	Hard Red Spring		10.13	8.46	53.7
10	(Unknown)		10.47	8.57	54.2
7	Hard Red Winter		10.86	9.19	50.4
24	Hard Red Spring		11.01	9.18	51.4
30	Hard Red Winter	Minnturki	11.05	9.27	49.5
23	Hard Red Spring		11.18	9.51	50.1
31	Hard Red Winter	"2614"	12.08	10.10	46.1
14	Hard Red Spring	Marquis	12.29	10.25	45.4
16	Hard Red Spring		12.39	10.50	44.8
8	White-Baart		12.52	10.37	47.9
20	Hard Red Spring		12.88	10.96	43.7
12	Hard Red Spring	Sturgeon	12.90	10.77	42.9
17	Hard Red Spring		13.00	11.14	42.1
11	Hard Red Spring	Progress	13.34	11.09	41.7
13	Hard Red Spring	Thatcher	13.55	11.38	40.7
18	Hard Red Spring		13.94	11.92	39.4
9	Hard Red Spring		14.39	12.36	38.6
25	Hard Red Spring		14.41	12.42	37.9
27	White		14.68	12.53	38.2
21	Hard Red Spring		14.82	12.83	37.1
22	Hard Red Spring		16.00	13.78	34.7
15	Hard Red Spring		16.26	14.22	33.9

From the curve in Figure 1 it is evident that the protein content can be predicted rather accurately from the light transmission values. Only three of the 34 flour samples show deviations from this relationship of more than 0.3% in terms of protein content, the mean deviation being 0.15% protein.
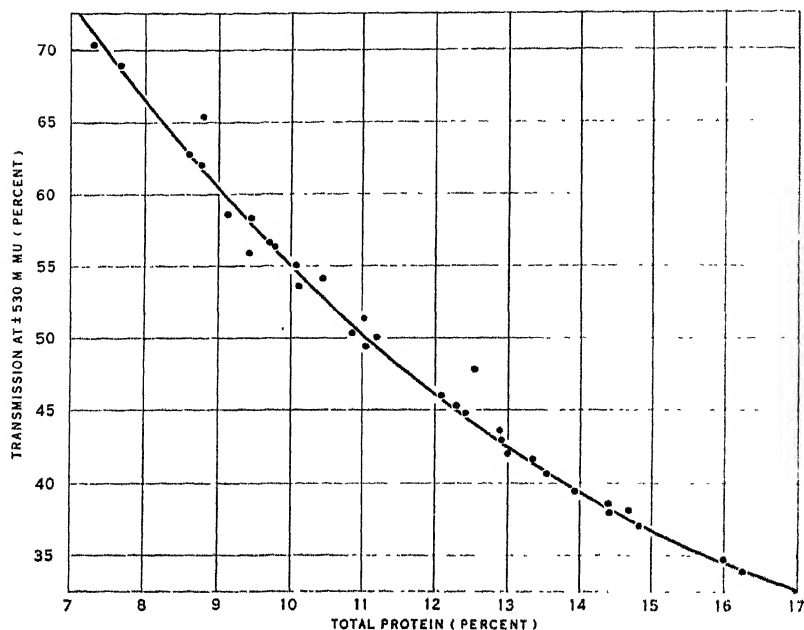
Fig. 1.   Relationship between total protein content and light transmission of protein suspension for 34 samples of wheat flour.

Since the turbidity produced in these suspensions is caused by the gluten proteins only, while the albumin and globulin remain completely dispersed, any variation in the normal ratio of gluten to nongluten protein will cause a deviation in the relationship between total protein content and light absorption. Theoretically, therefore, the light absorption values should be an even better index of gluten protein content than of total protein content.

To test this theory the protein peptizable in 5% potassium sulfate solution was determined on each of the 34 samples of flour under investigation, and by difference the protein not peptizable by the same salt solution was determined. This latter protein fraction may be considered an approximate measure of the gluten protein content, since the gluten proteins are not appreciably dispersed by 5% potassium sulfate. These values also are given in Table II and are shown graphically in Figure 2.

A comparison of Figures 1 and 2 shows a better relationship between light transmission and protein not peptizable with 5% potassium sulfate than between light transmission and total protein. The mean deviation was reduced to 0.13% protein and only one of the 34 samples deviated by more than 0.3% protein from the general relationship as
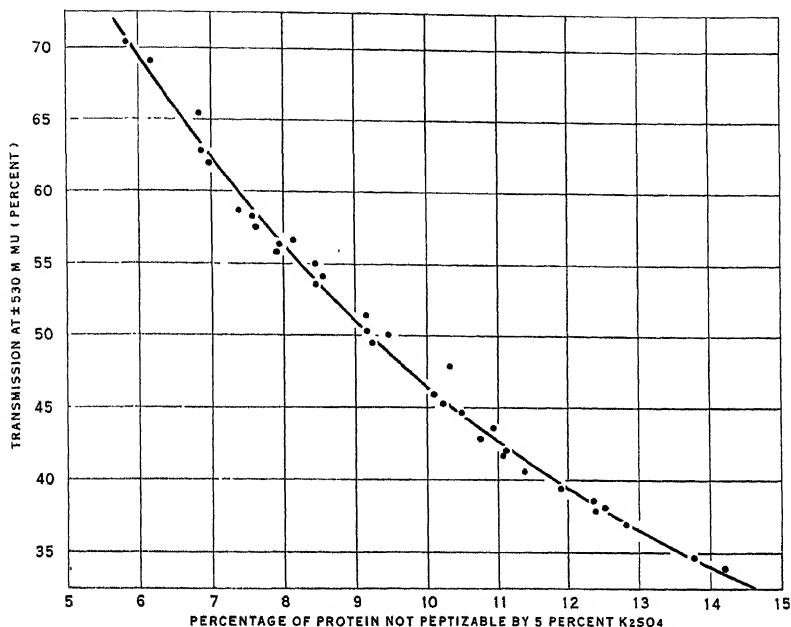
Fig. 2. Relationship between content of protein not peptizable by 5% K_2SO_4 and light transmission of protein suspension for 34 samples of wheat flour.

shown by the curve in Figure 2. It therefore appears that the photo-electric method is a slightly more exact measure of gluten protein content than of total protein content of wheat flour, and should therefore be a slightly better index of baking quality than the total protein content as determined by the Kjeldahl method.

A protein determination by the photometric method as described may be made in about the same length of time as by the Kjeldahl method, and may be made in considerably less time with but little sacrifice in accuracy by allowing only 15 minutes for the development of turbidity. For routine work the photometric method should prove considerably more rapid and less fatiguing to the analyst than the Kjeldahl method.

No table for converting photometric readings into protein percentages is presented herewith, since there is some question as to the uniformity of different makes of photometers, and since the results herein reported were obtained by the use of selected test tubes in the photometer instead of precision absorption cells. The use of such test tubes when well matched is highly desirable for routine work, and the results obtained are of the same order of accuracy as when precision cells are used. However, a conversion table based on such a

series of test tubes cannot be expected to apply to any other series of matched tubes. For the present, therefore, it is recommended that in the application of this photometric method each instrument be calibrated against the standard Kjeldahl procedure for a series of flour samples.

Conclusions

A photometric method for determining the protein content of wheat flour has been developed. The results are in good agreement with those obtained by the standard Kjeldahl procedure and appear to be a somewhat better measure of gluten protein than of total protein content.

The principal advantages of the photometric method for routine work should be the ease and rapidity with which a large volume of work can be handled with a minimum amount of equipment, and without the unpleasant features usually associated with a protein laboratory.

Efforts will be made to adapt the procedure to the determination of protein in wheat as well as flour. The success of this effort would greatly enhance the practical value of the method.

FACTORS THE BAKER SHOULD CONSIDER IN PREPARING THE YELLOW SPONGE CAKE

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The Altitude Laboratory of the Home Economics Section of the Colorado Experiment Station has continued a study of the baking of flour mixtures at various altitudes since 1926. In a recent bulletin W. E. Pyke and Gestur Johnson (1940) present certain data regarding preparing and baking yellow sponge cakes at different altitudes. One of the objectives of this study was to establish methods which would permit the true sponge cake to be prepared by means of large commercial mixing equipment. It seemed desirable also to improve, if possible, the general quality of sponge cake by a modification or improvement of methods of mixing used and by the adoption of a dependable formula with a large margin of safety so that the effect of small errors in measurement would be minimized. We believe that these objectives have been accomplished.

No attempt will be made in the present discussion to review the literature. Interested parties are referred to Barmore (1936) and to Pyke and Johnson (1940) for this information.

The commercial baker has used the true sponge cake or "full cream sponge" as it is sometimes called only to a rather small extent, because with the use of large mixing equipment it was difficult to produce it with satisfactory quality. At present prices for materials the cost of the ingredients for a true sponge cake of a size suitable for retail at 35 cents should not exceed 9 cents. This does not include cost of decoration, labor, packaging, and distribution. This relationship should be attractive, provided cakes of high quality can be reproduced with ease with present equipment.

From the beginning of this investigation it became apparent that when large mixing equipment was used the conventional method of mixing the true sponge cake could not be entirely adapted with satisfaction to the production of this type of cake. This was found to be due to the lack of stability of the mixed meringue as prepared by the

•——• Yolk Meringue (Hot Method)	×——× Whole Egg Meringue (Hot Method)
⊙——⊙ Yolk Meringue (Cold Method)	⊕——⊕ Whole Egg Meringue (Cold Method)
▲——▲ White Meringue (Cold Method)	⊖——⊖ Conventional Mixed Meringues
▫——▫ White Meringue (Hot Method)	
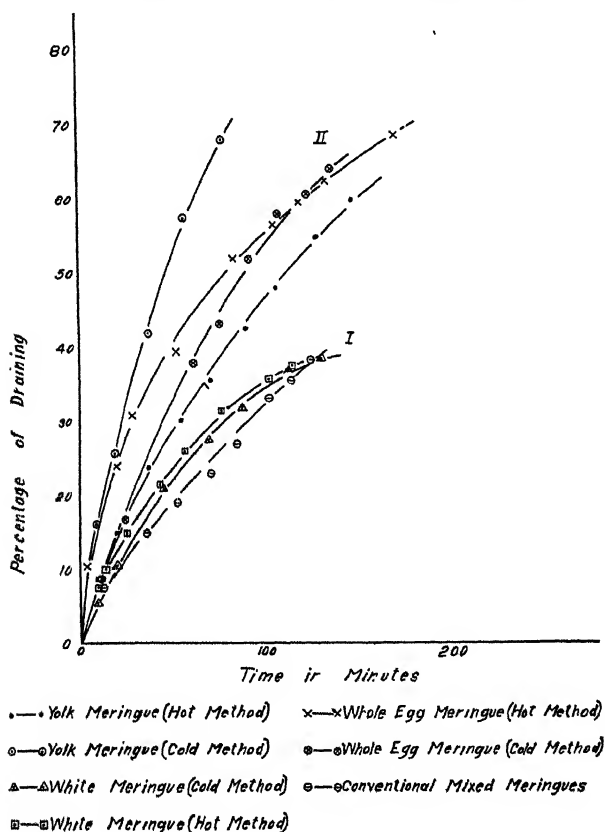
Fig. 1. Percentages of draining of meringues plotted against time.

conventional method and to the inability of the conventional sponge-
cake batter to stand the required amount of mechanical manipulation
or abuse.   Because of this situation a study was made of the properties
of various meringues containing egg yolk.   These properties were re-
lated to the properties of sponge-cake batters which could be prepared
therefrom by various methods of mixing.   Figures 1 and 2 show some
of these properties and relationships.

In Figure 1 the percentage of draining of a 36-g. sample of each
completed meringue through filter paper is plotted against time.   For
convenience of consideration these curves have been divided into two
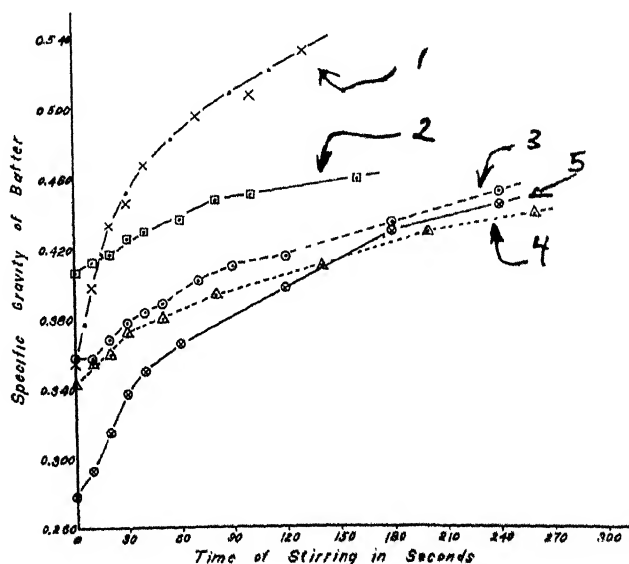
Fig. 2.   Effect of stirring at slow speed by means of the commercial type mixer upon sponge-cake
batters prepared by different methods of mixing.   Curve 1, conventional method of mixing, hand
folding.   Curve 2, hot single meringue method, flour added while meringue was hot.   Curve 3, cold
single meringue method of mixing.   Curve 4, hot single meringue method, flour added after cooling
meringue below 30°C.   Curve 5, meringue beaten by cold single method, flour folded in by hand to
point of origin, mechanical manipulation thereafter same as that of other batters.

groups.   Group I includes the more stable slow-draining meringues
and Group II the less stable rapid-draining meringues.   Briefly, the
method of preparation of these meringues was as follows.   The sugar,
salt, and cream of tartar were practically in solution in the egg magma
before whipping was started.   The egg substance and water used,
when water was an ingredient, had been mixed by beating a few seconds
at high speed before the solids were added.   The cold-method me-
ringues were prepared by whipping the mixture at high speed at room
temperature with the commercial-type mixer.   The hot-method me-
ringues were prepared similarly except that the three-quart bowl of the

mixer was immersed in a hot water bath at 80°C. during the beating. After beating, the bowl containing the hot meringue was immersed in cold water and the meringue was cooled quickly to 30°C., except in the case where the characteristics of the meringue while hot were being investigated (Curve 2, Fig. 2).

The composition of the meringue samples for the draining experiments and for the preparation of the corresponding batters follows. Egg-white meringues contained 150 g. sugar, 1 g. salt, and 4 g. cream of tartar to 210 g. egg white. Whole-egg meringues contained 134 g. sugar, 1 g. salt, 1 g. cream of tartar, and 30 cc. water to 192 g. whole egg (120 g. white and 72 g. yolk). Each yolk meringue contained 150 g. yolk, 130 g. sugar, 75 cc. water, and 1 g. each of salt and cream of tartar. For the conventional meringue one-half the sugar and the cream of tartar were whipped up with the white, and one-half the sugar and the water were beaten with the yolk. The resulting meringues were folded together either by machine or by hand as the purpose of the investigation demanded.

When these meringues were converted into cake batters, the following procedures were used. In the conventional method the flour was folded into the meringue during $3\frac{1}{2}$ minutes either by hand or mechanically. Mechanical folding in the formation of conventional sponge-cake batter produced a batter of too high specific gravity for the production of a satisfactory true sponge cake at low altitudes. For all other batters the flour was added in $6\frac{1}{2}$ minutes with continued stirring at slow speed by the commercial-type mixer. As a test of stability of the resulting batters, the stirring of these batters was continued in the commercial-type mixer at slow speed after the batter had been completed. At intervals the machine was stopped and the specific gravity of the batter determined. The batter used for the determination was returned to the mixing bowl and the stirring continued. The batter obtained from the yolk meringue was found to be too stable to obtain any appreciable effect on its lightness by this type of mechanical manipulation. For this reason there is no comparison of this type of batter in Figure 2.

It is apparent from a study of the curves represented in these two figures that the rapid-draining meringues produced far more stable batters. The batters produced by this improved method of mixing are sufficiently stable for production on the large scale with the use of heavy equipment. They are easily reproducible and yield low-cost cakes of excellent quality. That their texture is far superior to that of the conventional-type sponge cake is indicated in Figure 3. Other quality measurements also showed them superior.
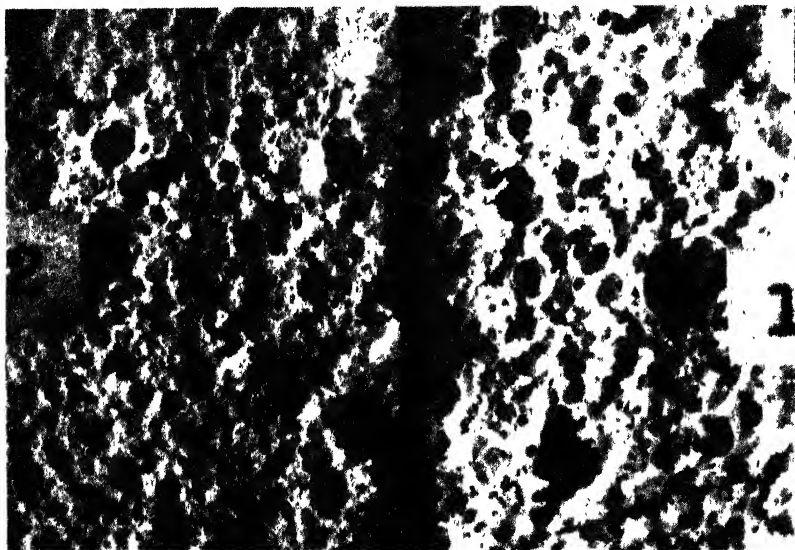
Fig. 3.   Sample 1 was prepared by the conventional method of mixing sponge cakes.   Sample 2 was prepared by the improved single-meringue method.   Magnification three diameters.

The basic sponge-cake formula used for 5,000 feet altitude was:

Egg white	120 g.	Cream of tartar	1 g.
Egg yolk	72 g.	Salt	1 g.
Cake flour	77 g.	Flavoring	2 cc.
Sugar	134 g.	Water	30 cc.

This formula produced a cake of medium size.   The sugar content was about the average of available sea-level recipes.   A study (Pyke and Johnson, 1940) indicated that a cake with a tensile strength of about 17 g. per square centimeter could be obtained at 5,000 feet altitude through the use of this formula when the leavening was correct and proper techniques were followed.

Evidence supporting the statement that these improved methods of mixing are superior to the conventional method is shown in Figure 4.   Throughout this figure the crosses represent cakes produced from conventional sponge-cake batters, the circled crosses represent cakes from a transition-type batter, and the circles represent cakes from batters mixed by the improved single-meringue method.   The transition-type batters were started like the conventional batter.   The white and yolk meringues were then mixed and beaten together at high speed.   The mixed meringues at first lost volume and then rapidly regained it.   The flour was added over 6½ minutes at low speed as with the improved single-meringue method.   Each cake was obtained from 380 g. of batter.   Baking conditions were controlled and were

the same throughout. All these cakes were mixed through all steps of preparation with the commercial-type mixer. It is evident that the improved single-meringue method of mixing is to be preferred when large mechanical equipment is to be used.

It has been common practice to omit measurement upon one of the ingredients of the true sponge cake. This ingredient is the leavening
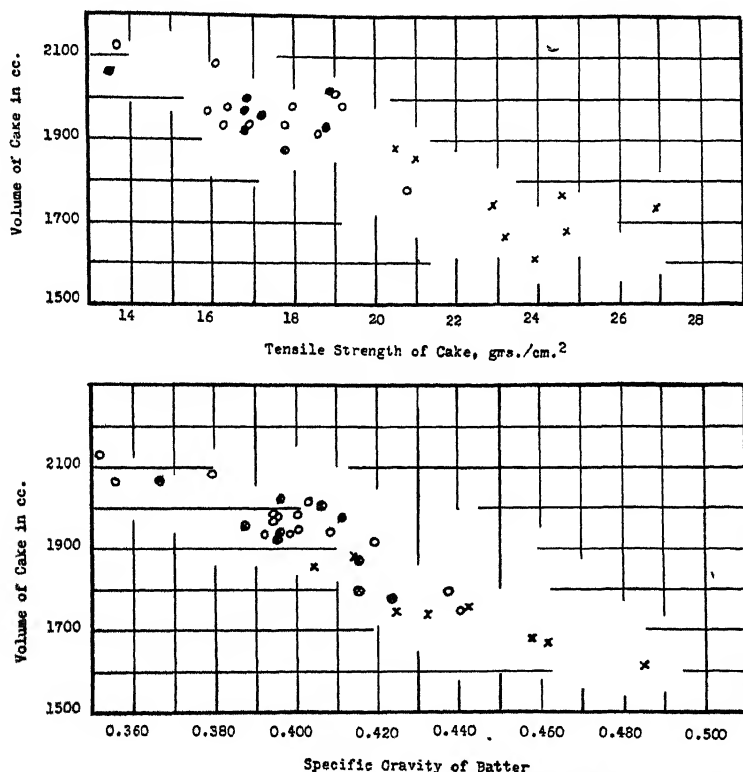
Fig. 4. A comparison of three methods of mixing the true sponge cake when commercial-type mixing equipment is used throughout. The crosses represent batters and cakes prepared by the conventional method. Circles represent batters and cakes prepared by the cold single meringue method. Filled circles represent a transition-type batter. Lack of stability of the conventional-type batter on the machine caused the production of a heavy batter of little practical value. The other methods are suitable for machine use.

air. It is apparent that such an omission could prove ruinous if large-scale production were attempted. Without control of the leavening a standardized product is impossible. With this control, good technique, and standard equipment, a highly attractive, readily reproducible product is possible. The leavening agent in the true sponge cake can be measured indirectly by the determination of the specific gravity or specific volume of the batter. Figure 5 shows the relationship between
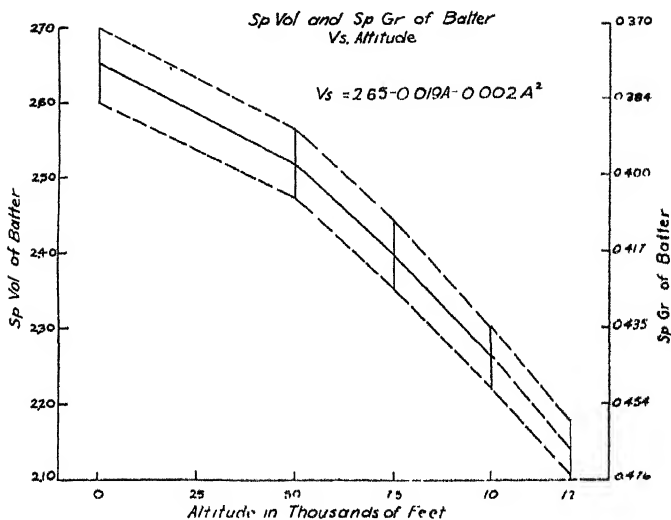
Fig. 5.   As altitude increases the specific gravity of the sponge-cake batter should increase if cakes of comparable volume and texture are to be obtained.

these values for proper leavening effect at different altitudes.   This curve applies directly to the cake formulas to be given later.   It will apply to other true sponge-cake formulas, but the intercepts may be altered in such cases.

Certain other factors are very important when large equipment is to be used in mixing the true sponge cake.   Fisher (1936) has called attention to the interrelationships that exist between size and type of mixing equipment and the results obtained with the A.A.C.C. baking method.   In the preparation of sponge-cake batters the interrelationship of size of bowl and whip to the size of the batch and speed is most important.   Underloading or overloading a bowl of any particular size may lead to costly and disappointing results.   The effective whip speed is definitely related to the size of the bowl.   Within limits as the size of bowl and batch increase, the speed of the whip should decrease.   If, then, bowls of varying sizes are to be used on the same machine, provision for a multiplicity of speeds should be available.

The bakery engineer has made steady improvement of available equipment and is to be commended on the remarkable progress that has been made.   In spite of this, however, the available speeds upon a machine may not suit the case in hand.   When two speed ratios are available, one of which is too violent to complete the meringue to its desired lightness, and the other too slow, our experience has shown that a meringue of the desired lightness can be formed by whipping with the higher speed until its maximum effect has been obtained and then

shifting to the next lower speed. This takes longer than the correct speed ratio, but it works. The important thing is to get the whole-egg or yolk meringue light enough. A specific gravity of 0.22 for the meringue at 20° to 30°C. is safe. A lighter meringue will not be detrimental.

Bakers for a long time have depended upon some fixed time interval for the control of mixing processes. While this is safe with butter-type cakes, its safety is doubtful with the sponge cake, both the angel food and yellow sponge. Eggs of different age and quality form meringues at very different rates. If time is the criterion used to determine when the meringue is completed, the cake batters formed from these meringues will have quite different characteristics. That the cakes from such batters vary considerably is evidenced by the observations of numerous investigators (Barmore, 1936; Hunt and St. John 1931; Glabau, 1940). Barmore (1936) has shown that if the egg-white foams are beaten to the same specific gravity identical cakes result. An implied proviso to this statement is that the egg whites should not vary too greatly in quality. This qualification arises from the fact that egg-white foams of the same specific gravity but varying considerably in quality shrink differently when the flour is added. The shrink in the oven and after baking also is quite different; so eggs of average or higher quality should be used. With the yellow sponge cake, prepared by the improved method we have outlined, wide and notable differences in rate of meringue formation with eggs from different grades need not affect the lightness of the finished batter, if the procedure outlined is followed. Specific gravity determinations and not time should be used to indicate when the batter is completed, if high quality, uniformity, and reasonable economy are to follow.

Another relationship with which the baker is familiar is of considerable importance in the baking of the yellow sponge cake. This is pan type, size, and shape. Their relationship to the quantity and lightness of the batter is apparent. Since sponge-type cakes are supported considerably during cooling by their grip upon the surface of the pan, the span between surfaces should not be too great, or contraction will produce a dip midway across the span. Related factors here are the elasticity and tensile strength developed within the cake during baking. Within the temperature range used, elasticity and tensile strength for any given formula are directly proportional to the internal temperature attained during baking. This approximates 100°C. at sea level and drops about 1°C. for each 1,000 feet above sea level in altitude. Near the hot pan surface this temperature is considerably exceeded. The greatest strength of the structure is developed adjacent to the pan surface, provided the sugar-water ratio has been correct for

the pressure conditions under which the cake was baked. If the sugar-water ratio has been too high, a sticky semisolid layer arises next to the pan. As the cake cools, this sticky layer fails to hold. Various stages of collapse due to contraction may result from such conditions. The greater portion of the water evaporation occurs in this highly heated layer. Sugar solution diffuses into this layer to replace continuously the water evaporating. If the relative sugar concentration becomes too great in this layer, neither starch nor egg substance sets properly and a sticky layer results.
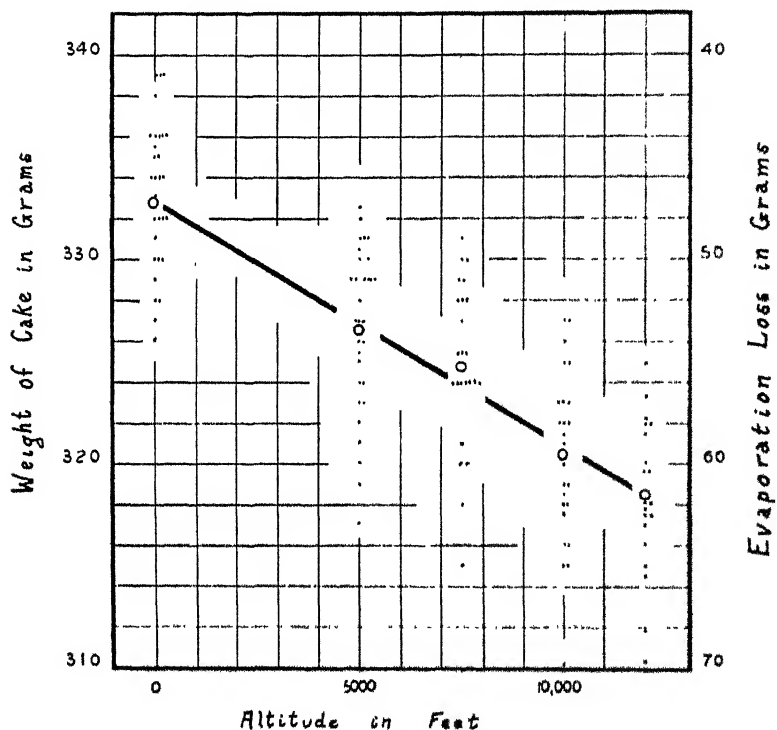
Fig. 6. Weight losses when 380 g. of batter are baked at different altitudes. The water content of sponge-cake batters should be increased as the altitude increases, to correct for increased evaporation.

Since the rate of evaporation increases as the atmospheric pressure decreases, more water per gram of sugar must be present at high altitudes than at low altitudes. Figure 6 indicates how this rate of evaporation is changed as the altitude increases. To provide a reasonable margin of safety, the water correction for the cake formula should exceed slightly the amount indicated. When the water corrections indicated in the sponge-cake formulas given later are made, the sugar solution within the cake will vary between 52% and 54% sugar over

the altitude range from 0 to 12,000 feet, if we assume uniform distribution of the sugar in the water. These variations arise largely from practical considerations in adjusting the recommended measurements to reasonably convenient values. It is possible to balance sponge-cake batters higher in sugar content which may be used successfully over this altitude range, but organoleptic tests upon cakes from such batters did not indicate any appreciable increase in the sweetness of the product to the taste. By other quality measures these cakes still higher in sugar content were not superior but were inferior to the cakes with the sugar content chosen. Economy considerations also favor the sugar content selected. In Figure 7 we see the results one may expect to
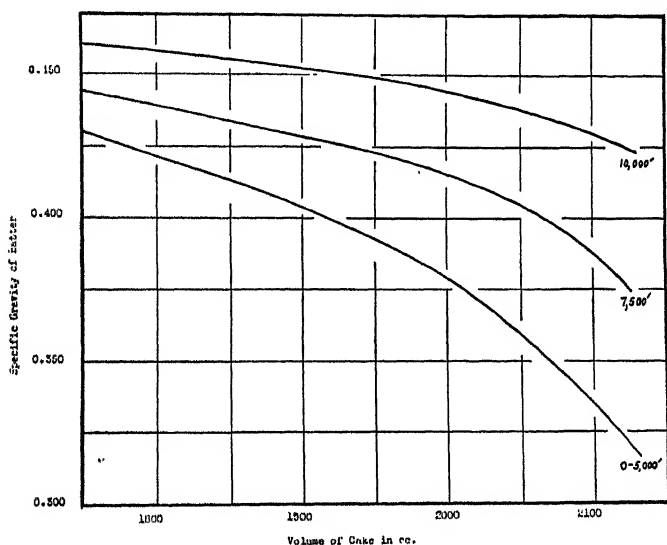
Fig. 7. A nomograph showing the volume expected from 380-g. samples of sponge-cake batter of different specific gravities when baked at different altitudes.

obtain with the use of the whole-egg sponge cake formula at different altitudes, when 380 g. of batter having different specific gravities are used. The standard deviations of the volumes of cakes for a variation of 0.01 in specific gravity in the 1,950-cc. range at 5,000 feet altitude was found to be 40 cc. in a series of 14 cakes. The error included the effects of the natural fluctuations in atmospheric pressure at 5,000 feet as well as other sources of error. The standard deviations in the 2,050-cc. range ran slightly lower. We found no difficulty from a practical standpoint in controlling the specific gravity to a variation of 0.003. It is evident from Figure 7 that a more precise control of the specific gravity of the batter should occur at high altitudes than at low altitudes to obtain the same uniformity in volume.

A study of the effect of altitude upon the tensile strength of sponge cakes is shown in Figure 8. The procedure used in arriving at these values is described elsewhere (Pyke and Johnson, 1940). The equation given, $T = 19.9 - 0.03A - 0.114A^2$, applies to cakes of a specific gravity of 0 168. It will be noted that these sponge-cake formulas yield more tender sponge cakes than have heretofore been recommended. They are of much higher quality and have better keeping
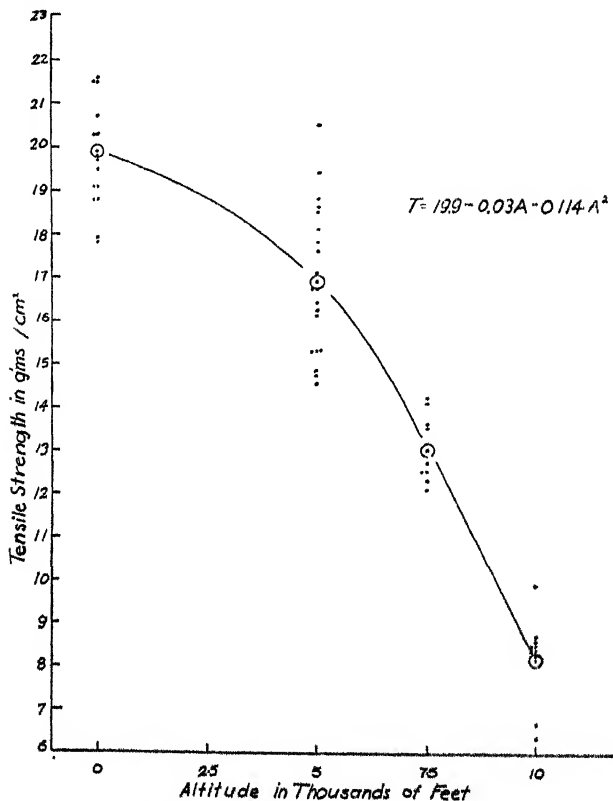
Fig. 8. Relation of tensile strength to altitude.

characteristics than those of heavier, tougher cakes. The improved method of mixing described produces a texture (Fig. 3) superior to the conventional method so that taste impressions are improved, probably because of the accompanying more velvety, more moist sensation on the tongue.

The formulas and procedures for yellow sponge cakes are taken from the Colorado Experiment Station Technical Bulletin 27 (Pyke and Johnson, 1940).

WHOLE-EGG SPONGE CAKE FORMULA

Ingredients	Altitude	Metric formula	Commercial formula		Housewife's formula (volume measure)		
	Ft.	Grams	Lbs.	Oz.	Cups	T.	t.
Cake flour	0 to 5,000	77	2	0	$\frac{3}{4}$+	1+	2
sifted once	7,500	85	2	4	1−	1−	2
before measuring	10,000	95	2	8	1+	1+	0
	12,000	105	2	12	1+	2+	2
Water[1]	0	20	0	8	—	1+	1
	2,500	25	0	10	—	1+	2
	5,000	30	0	$12\frac{1}{2}$	—	2+	0
	7,500	30	0	$12\frac{1}{2}$	—	2+	0
	10,000	35	0	14	—	2+	1
	12,000	40	1	0	—	2+	2
Egg white	Any	120	5 pounds whole eggs		4 medium-sized eggs		
Egg yolk	Any	72					
Sugar	Any	134	3	8	$\frac{2}{3}$+	0+	0
Salt	Any	1	0	$\frac{1}{2}$	0+	0+	$\frac{1}{4}$
Cream of tartar	Any	1	0	$\frac{1}{2}$	0+	0+	$\frac{1}{4}$
Vanilla [1]	Any	2 cc.	0	1	0+	0+	$\frac{1}{2}$
		Grams			Ounces		
Correct weight of 1	0	87 to 91			3 to $3\frac{1}{5}$		
cup (net) of batter for	2,500	90 to 93			$3\frac{1}{8}$ to $3\frac{1}{4}$		
proper leavening	5,000	92 to 96			$3\frac{1}{4}$ to $3\frac{3}{8}$		
	7,500	97 to 100			$3\frac{7}{16}$ to $3\frac{1}{2}$		
	10,000	103 to 106			$3\frac{5}{8}$ to $3\frac{3}{4}$		
	12,000	109 to 112			$3\frac{13}{16}$ to $3\frac{15}{16}$		

[1] It is often desirable to include lemon juice and grated lemon peel as a flavor in sponge cake in addition to the small amount of vanilla advised. The amount of water to be added should be corrected for the amount of lemon juice to be added. Five cc. or 1 teaspoon of juice and 1 tablespoon of freshly grated peel should be added at the end of the mixing period for the metric and housewife's formula. For the commercial formula $2\frac{1}{2}$ ounces of juice and 1 ounce of grated peel should be used.

Procedures

A. Single Meringue Method of Mixing

Cold method.—Equipment recommended for this method of mixing is an efficient mechanical mixer with a wire whip. The egg whites, yolk, and water required are cut together with the beater to mix them thoroughly. The sugar, salt, cream of tartar, and vanilla are dissolved in the egg, and the mixture is beaten until the meringue is very light and just fails to stand in a peak. The mixer is changed to slow speed and the cake flour added slowly over a period of six minutes. The bowl is thoroughly scraped down, the lemon juice and grated peel added if desired, and mixing continued one-half minute at slow speed. The batter is then weighed in a standard tared cup. If necessary (this is always the case at high altitudes), stirring is continued at slow speed until a cup of batter gives the required net weight. Baking should be

in an unlined, ungreased tube pan with removable bottom. Cakes should be inverted to cool. For jelly roll the heavier of the recommended weights should be used for each altitude. The jelly roll batter should be baked in a large, flat, lined pan.

Hot method.—The hot method is recommended for hand equipment with double Dover beater, small mixing equipment, and large commercial equipment. This method is the same as the cold method except that the bowl containing the egg mixture after the solids have dissolved is placed in a pan of hot water (80°C. or 175°F. for the greatest effect) during the beating period. The meringue comes up much more rapidly by the hot method. When the meringue forms a soft peak, the bowl is placed in cold water and the contents cooled with occasional stirring. The cake flour is added as directed under the cold method. The batter gives a larger volume by the hot method than by the cold method. The heavier of the net weights for a cup of batter is to be preferred for each altitude.

B. Conventional Method of Mixing

Equipment recommended is either hand equipment or a combination of hand and mechanical equipment. The whites and yolks are beaten up separately. The water required, the salt, and the vanilla are added to the yolks and mixed by a few seconds of beating. One-half of the sugar required for the formula is added and stirred until mostly dissolved. This mixture is beaten until it forms a soft-peaked meringue. The whites with the cream of tartar and one-half the sugar are beaten to a meringue that just peaks but is not too stiff. The two meringues are folded together. The flour is then folded into the mixed meringue. If lemon juice and peel are desired they are also folded in. Folding and mixing the batter are continued until the required leavening density is reached as determined by the weight of a cup of batter. This method of mixing does not give as desirable a cake as is obtainable by procedure *A*. The mixed meringues and the finished batter are very sensitive to mechanical abuse. Baking directions are the same as for the cake mixed by the single meringue method.

BAKING TIME AND TEMPERATURES

Altitude	Degrees C.	Degrees F.	Time in minutes [1]
0 and 2,500	165	330	50–55
5,000 and 7,500	171	340	55–60
10,000 and 12,000	177	350	63–65

[1] Baking time depends upon shape of pan and its size. The time recommended is for a batter of 380 g. or 13⅜ oz. (the 4-egg cake) in a pan permitting the height of the cake to approximate the distance from the tube to the side of the pan, measured at the top of the cake. A flat sheet for jelly roll or smaller batches should be baked for shorter periods of time. In a controlled oven, the top heat should be low and the bottom heat high.

YOLK SPONGE CAKE FORMULA.[1]

Ingredients	Altitude	Metric formula	Commercial formula		Housewife's formula (volume measure)		
	Ft.	Grams	Lbs.	Oz.	Cups	T.	t.
Cake flour	0 to 5,000	100	2	0	$1+$	$2+$	0
sifted once be-	7,500	110	2	3	$1\frac{1}{4}+$	$0+$	2
fore measuring	10,000	130	2	6	$1\frac{1}{2}-$	$0-$	2
	12,000	130	2	9	$1\frac{1}{2}+$	$2+$	0
Water	0	70	1	6	$\frac{1}{4}+$	$0+$	2
	2,500	75	1	8	$\frac{1}{4}+$	$1+$	0
	5,000	85	1	11	$\frac{1}{4}+$	$1+$	2
	7,500	90	1	$12\frac{2}{3}$	$\frac{1}{4}+$	$2+$	0
	10,000	100	2	0	$\frac{1}{4}+$	$2+$	2
	12,000	110	2	3	$\frac{1}{4}+$	$3+$	1
Egg yolk	Any	150	3	0	8 egg yolks		
Sugar	Any	134	2	11	$\frac{2}{3}+$	$0+$	0
Salt	Any	1	0	$\frac{1}{2}$	$0+$	$0+$	$\frac{1}{4}$
Cream of tartar	Any	1	0	$\frac{1}{3}$	$0+$	$0+$	$\frac{1}{4}$
Vanilla	Any	2 cc.	0	1	$0+$	$0+$	$\frac{1}{2}$

		Grams	Ounces
Correct weights of 1	0	91 to 93	$3\frac{1}{8}$ to $3\frac{1}{4}$
cup (net) of batter for	2,500	93 to 95	$3\frac{1}{4}$ to $3\frac{3}{8}$
proper leavening	5,000	98 to 100	$3\frac{7}{16}$ to $3\frac{1}{2}$
	7,500	103 to 106	$3\frac{5}{8}$ to $3\frac{3}{4}$
	10,000	112 to 115	$3\frac{15}{16}$ to $4\frac{1}{16}$
	12,000	118 to 120	$4\frac{1}{8}$ to $4\frac{1}{4}$

[1] This cake is far more tender than the usual sponge cake. It requires more careful handling, but its high quality recommends it. The baking temperature for the yolk sponge cake is the same as for the whole-egg sponge cake. The baking time should be increased by 10 minutes. *Top heat should not be used.*

Summary

New methods of mixing have been investigated and described. They have been adapted for use with large commercial equipment as well as with small equipment. These new methods produce sponge cakes which are superior to the conventional-type sponge cake.

Factors which will concern the commercial baker when he produces the true sponge cake have been discussed. If the recommendations are followed, it should be possible to produce high-quality, low-cost cakes of the true sponge type upon a large scale.

The leavening of true sponge cakes (angel food and yellow sponge) should be controlled by specific gravity determinations upon the batter. Time of beating as a factor to control this ingredient is not dependable.

Formulas for the preparation of yellow sponge cakes at various altitudes have been given. Interpolation between the intervals of 2,500 feet is permissible.

The formulas for yolk sponge cakes adjusted to various altitude conditions yield cakes which compare to butter cakes in tenderness. Their quality is high. They require a longer baking period than the whole-egg sponge.

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A PRELIMINARY SURVEY OF THE VITAMIN B₁ CONTENT OF AMERICAN CEREALS

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Of all the vitamins known to man, vitamin B_1 appears to be most closely related to carbohydrate metabolism. Also, of all the foods known to man, the cereals are the chief source of carbohydrates. Furthermore, the carbohydrates provide the major part of the calorie intake of the average human diet. It is a generally accepted theory that the utilization of each gram of carbohydrate requires a specific amount of vitamin B_1.

The determination of vitamin B_1 in cereals has heretofore been somewhat difficult because of the relatively low B_1 content. The fermentation method for B_1 determinations developed at our laboratories [1] is, however, very convenient for such measurements. With this method we have analyzed some 130 grains in a preliminary survey designed to determine the average B_1 content, the range of values, and the influence of variety.

The method of analysis has already been published, and in the present communication only the preparation of the sample will be described. The seed was ground in a Seck mill at the finest setting.

One to two grams of the meal were weighed into a 100-ml. volumetric flask and about 50 ml. of water added. Sufficient sulfuric acid was added to make the reaction blue to congo red paper and then it was heated in flowing steam for 20 minutes. When cool, the solution was neutralized to neutral litmus with NaOH and made up to volume. Aliquot fractions of the well stirred suspension were transferred to the reaction or fermentation bottles by a fast-flowing pipette. It is sometimes more convenient to take aliquots with a graduated cylinder. The reproducibility of the technique is reasonably high; checks do not usually differ by more than 5%.

The data which we have obtained cannot be considered as being absolutely representative of the various grains. Too few samples have been examined as yet. The wheats, for example, comprise a random group, including durum, and the results are no more representative of soft than of hard wheats. However, the results of this preliminary survey may be of interest in view of the observed variability in the vitamin content, which shows the necessity for further research.

Results

Thirty-one wheat samples in all were assayed.[2] The data are given in Table I. The values ranged from 4.2 to 7.3 γ per gram. The average thiamin content is 5.6 γ per gram. This average is of doubtful significance since the samples make a heterogeneous group. Insufficient data have been gathered, as yet, to draw any conclusions regarding the exact effect of climate, soil, etc., but since there are significant differences throughout the group one may assume that these factors are not without effect.

The assays of 37 barleys are given in Table II. With a range of 3.8 to 9.2 they averaged 6.2 γ thiamin per gram. The great spread in values is evidenced by the fact that eight samples were higher than 7.2.

In the case of the barleys we were fortunate in having a certain amount of data on grades and strains. Table III shows how the thiamin content of barley appears to vary with the grade. The No. 1 grade which has the largest kernel size has the lowest average thiamin content. These differences are probably related to the distribution of thiamin between the various parts of the seed; i.e., the No. 1 grade may have a larger proportion of a low thiamin-bearing constituent.

Table IV shows that three samples of Oderbrucker averaged 6.17 against 5.55 γ thiamin per gram for four samples of Wisconsin 38.

TABLE I
WHEAT ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
9F175 A	Spring—Mindum Durum	St. Paul, Minn.	7.1
9F175 B	Spring—Thatcher	" " "	7.3
9F175 C	Winter—Minturki	" " "	6.9
9F208 A	Early Blackhull C.I. No. 8856	Manhattan, Kan.	4.5
9F208 B	Kanred C.I. No. 5146	" "	5.0
9F208 C	Blackhull C.I. No. 6251	" "	5.2
9F208 D	Turkey C.I. No. 1558	" "	4.5
9F208 E	Tenmarg C.I. No. 6936	" "	5.7
9F208 F	Kawvale C.I. No. 8180(8186?)	" "	4.5
9F208 G	Clarkan C.I. No. 8858	" "	6.7
9F208 H	Chiefkan C.I. No. 11754	" "	4.7
9F208 I	Nebred C.I. No. 10094	" "	6.0
9F208 J	Cheyenne C.I. No. 8885	" "	5.2
9F208 K	Kharkof C.I. No. 1442	" "	4.5
9F208 L	Oro X Tenmarg C.I. No. 11673	" "	6.0
9F088 A	Spring 13.5% prot.	Chicago, Ill.	5.9
9F088 B	White 14.4% prot.	" "	6.1
9F088 C	No. 1 Amber Durum 11.9% prot.	" "	5.9
9F088 D	No. 1 Hard Winter 14.5% prot.	" "	6.7
9F088 E	No. 1 Hard White 13.5% prot.	" "	6.1
9F088 F	White	" "	6.1
9F088 G	Minnesota Winter 13.5% prot.	" "	5.9
9F088 H	Minnesota Winter 11.6% prot.	" "	5.5
9F088 I	Red Durum 14.5% prot.	" "	6.3
9F088 J	Montana Winter 15.0% prot.	" "	6.0
9F088 K	Montana Spring	" "	5.8
0F144 A	Nittany	East Lansing, Mich.	5.2
0F144 B	Baldrock	" " "	4.2
0F144 C	American Banner	" " " "	4.5
0F144 D	Trumbull	" " " "	4.7
0F144 E	Purkoff	" " " "	5.1

With regard to the specific effect of any given region little can be said except to conclude that such effects do exist. Further work, which will probably be done by those most interested, should give the answer.

Twenty-three samples of corn (Table V) averaged 5.34 γ per gram. With the exception of one sample at 8.0, all the samples fell between 4.1

TABLE II

BARLEY ASSAYS

Lab. No.	Name	Obtained from			Thiamin, γ per gram
9F137 A	Club Mariout	Hays, Kan.			6.3
9F137 B	Spartan	"	"		7.2
9F137 C	Vaughn	"	"		7.1
9F137 D	Stavropol	"	"		8.7
9F137 E	Flynn	"	"		6.5
9F137 F	Trebi	"	"		8.2
9F137 G	Atlas X Vaughn	"	"		7.7
9F137 H	Atlas	"	"		7.5
9F137 I	White Smyrna	"	"		7.1
9F137 J	Lico	"	"		7.1
9F137 K	Franklin (Oderbrucker)	"	"		6.2
9F176 A	Wisconsin No. 38	St. Paul, Minn.			6.5
9F176 B	Velvet	"	"	"	7.5
9F176 C	Improved Manchuria	"	"	"	9.2
0F141 A	Spartan	East Lansing, Mich.			5.7
0F141 B	Wisconsin No. 38	"	"	"	5.5
9F087 A	"D" South Dakota	Chicago, Ill.			6.1
9F087 B	Illinois	"	"		6.1
9F087 C	"D" North Dakota	"	"		6.1
9F087 D	"D" Wisconsin	"	"		5.6
9F087 E	No. 2 North Dakota	"	"		5.6
9F087 F	No. 1 Minnesota	"	"		5.1
9F087 G	No. 2 Minnesota	"	"		5.6
9F087 H	No. 1 Michigan	"	"		5.1
9F087 I	No. 2 Michigan	"	"		5.3
9F087 J	No. 1 North Dakota	"	"		4.7
9F087 K	Red River Valley (N.W. Minnesota)	"	"		5.3
9F087 L	Oderbrucker (Wisconsin)	"	"		6.6
9F087 M	No. 1 South Dakota	"	"		5.8
9F087 N	Pedigree 38 (Wisconsin)	"	"		5.5
9F087 O	No. 1 Wisconsin	"	"		5.1
9F087 P	No. 2 Wisconsin	"	"		5.1
9F087 Q	No. 1 Hanna (2 row) (Oregon)	"	"		3.8
9F087 R	No. 2 Hanna (2 row) (Oregon)	"	"		4.7
9F087 S	No. 2 Iowa	"	"		5.0
9F195	Oderbrucker	Madison, Wis.			5.7
9F196	Wisconsin 38	"	"		4.7

TABLE III
RELATION OF BARLEY GRADES TO THIAMIN CONTENT

	Grade		
Sample marked	No. 1	No. 2	D
	γ per g.	γ per g.	γ per g.
Minnesota	5.1	5.6	--
North Dakota	4.7	5.6	—
Michigan	5.1	5.3	—
South Dakota	5.8	—	6.1
Wisconsin	5.1	5.1	5.6
Oregon	3.8	4.7	--
Average	4.93	5.26	5.85

TABLE IV
THIAMIN CONTENT OF TWO BARLEY STRAINS

Sample obtained from	Wisconsin No. 38	Oderbrucker
	γ per g.	γ per g.
Illinois	5.5	6.6
Wisconsin	4.7	5.7
Minnesota	6.5	—
Michigan	5.5	—
Kansas	—	6.2
Average	5.55	6.17

TABLE V
CORN ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
9F177 A	Minnesota No. 13	St. Paul, Minn.	5.6
9F177 B	Rustler	" " "	5.8
9F207 A	Colby Yellow Cap (Kan.) C.I. No. 2894	Manhattan, Kan.	5.0
9F207 B	Cassel White (Kan.) C.I. No. 2891	" "	6.1
9F207 C	Commercial White (Kan.) C.I. No. 2881	" "	5.5
9F207 D	Midland (Kan.) C.I. No. 2877	" "	5.1
9F207 E	Hays Golden (Kan.) C.I. No. 2875	" "	6.0
9F207 F	Reid (Kan.) C.I. No. 2873	" "	5.5
9F207 G	Pride of Saline (Kan.) C.I. No. 2867 ?	" "	6.0
9F207 H	Yellow Selection No. 1 C.I. No. 2768	" "	5.3
9F207 I	Iowa 939 (Nebraska) C.I. No. 2887	" "	4.6
9F207 J	US 13 (Ohio) C.I. No. 2885	" "	4.1
9F207 K	US 44 (Illinois) C.I. No. 2876	" "	4.6
0F193 A	Pickett	East Lansing, Mich.	4.1
0F193 B	Duncan	" " "	5.8
0F193 C	M.A.C. Yellow Dent	Washington, D.C.	4.7

TABLE V—(*Continued*)

Lab. No.	Name	Obtained from	Thiamin, γ per gram
0F193 D	Northwestern Dent	Washington, D.C.	5.0
0F194 A	Gehu Yellow Flint (North Dakota)	" "	8.0
0F194 B	Dakota White Flint (North Dakota)	" "	6.0
0F194 C	Golden King (Iowa)	" "	6.0
0F194 D	Krug (Iowa)	" "	4.8
0F194 E	Whatley Prolific (Georgia)	" "	4.5
0F194 F	Garrick Prolific (South Carolina)	" "	4.7

TABLE VI

OAT ASSAYS

Lab. No.	Name	Obtained from	Thiamin, γ per gram
0F174 A	Rusota	St. Paul, Minn.	9.6
9F174 B	Minrus	" " "	10.0
9F174 C	Gopher	" " "	10.3
9F174 D	Anthony	" " "	8.2
9F174 E	Iogold	" " "	9.7
9F206 A	Fulton C.I. No. 3327	Manhattan, Kan.	8.0
9F206 B	Kanota C.I. No. 839	" "	8.2
9F206 C	Brunker C.I. No. 2054	" "	6.0
9F206 D	Columbia C.I. No. 2820	" "	7.5
0F142 A	Iowa 444	East Lansing, Mich.	6.5
0F142 B	Huron	" " "	7.0
0F142 C	Markton	" " "	6.0
0F142 D	Iogold	" " "	6.0
0F142 E	Worthy	" " "	6.0
0F142 F	Wolverine	" " "	7.0
0F195 A	Iowar (Iowa) C.I. No. 847	Washington, D.C.	8.8
0F195 B	Cornellian (Idaho) C.I. No. 1242	" "	5.2
0F195 C	Richland (Idaho) C.I. No. 787	" "	4.8
0F195 D	Lenroc (Idaho) C.I. No. 3205	" "	5.0
0F195 E	Silvermine (N. Dak.) C.I. No. 659	" "	5.7
0F195 F	Fulghum (Idaho) C.I. No. 708	" "	5.7

and 6.1.   No significant difference could be observed between the white and yellow varieties.

The assay of 21 samples of oats (Table VI) showed the wide range of 4.8 to 10.3 γ per gram with an average of 7.2.   A considerable number of samples analyzed 8.0 and above.

TABLE VII

RYE ASSAYS

Lab. No.	Name	Obtained from		Thiamin, γ per gram
9F178	Dakold	St. Paul, Minn.		5.7
0F143	Rosen	East Lansing, Mich.		4.0
0F196 A	Dakold (Nebraska)	Washington, D.C.		5.7
0F196 B	Wisped (Wisconsin)	"	"	4.7
0F196 C	Rosen (Michigan)	"	"	4.5
0F196 D	Balbo (Virginia)	"	"	5.0
0F196 E	Rimpau (Virginia)	"	"	5.0
0F196 F	Raritan (Virginia)	"	"	4.5
0F196 G	Abruzzi (Virginia)	"	"	4.1
0F196 H	Smooth Neck (Virginia)	"	"	5.0

Ten rye samples (Table VII) gave the relatively narrow range of 4.0 to 5.7 with an average of 4.84 γ per gram. The two samples of Dakold rye, one from Minnesota and the other from Nebraska, gave the same result, whereas two samples of Rosen rye, both from the state of Michigan, differed somewhat.

Table VIII shows the assay of five buckwheats. The two rye buckwheats are considerably higher than the others, which is probably related to the fact that they represent a different species of the herb. Three millets (Table IX) averaged 7.23 γ per gram.

TABLE VIII

BUCKWHEAT ASSAYS

Lab. No.	Name	Obtained from		Thiamin, γ per gram
0F197 A	Wing Emarginatum (Virginia)	Washington, D.C.		5.0
0F197 B	Japanese (Virginia)	"	"	4.7
0F197 C	Silverhull (Virginia)	"	"	4.2
0F197 D	Rye-Buckwheat Sel 37 (Virginia)	"	"	7.5
0F197 E	Rye-Buckwheat (Virginia)	"	"	8.5

TABLE IX

MILLET ASSAYS

Lab. No.	Name	Obtained from		Thiamin, γ per gram
0F198	Yellow Manitoba proso (Colorado)	Washington, D.C.		8.2
0F211 A	Common F.C. No. 22,637	"	"	7.5
0F211 B	German (Tenn.) F.C. No. 22,636	"	"	6.0

Summary

From a preliminary survey of the vitamin B_1 (thiamin) content of American cereals it is possible to draw the following conclusions:

There are significant differences in the thiamin content of the various cereals.

There are significant differences in the thiamin content of various strains of the same cereal, and there are indications that regional differences may affect the thiamin content of a single strain.

In view of the spread in values which is observed in most of the cereals it is not possible to evaluate a diet or food mixture containing cereals without analysis of the ingredients.

THE NATURE OF THE LIBERATION OF BOUND BARLEY AMYLASE AS EFFECTED BY SALT SOLUTIONS [1]

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The early observations of a phenomenal increase in the quantity or activity of amylase enzymes during barley germination in the production of malt have since been repeatedly confirmed. Further, the nature of this increase in activity of the amylase enzymes has been the subject of much speculation and investigation. Early observers thought that it was due to a production of new enzymes, that is, synthesis. Subsequent, however, to the important discovery of latent or bound enzymes by the English investigators Ford and Guthrie (1908), this trend in thought underwent change.

Although the literature contains no specific and practical definition of the concept of a bound or latent amylase, it can be assumed from the works of various investigators that the expression refers to that portion of the total quantity of amylase present in a given barley or malt sample which cannot be extracted by distilled water. In other words, it is the quantity of enzyme that represents the difference between the "total" quantity as extracted by special methods and the "free" quantity that may be extracted by distilled water.

While at times the literature has used the terms "bound" and "latent" interchangeably, it is felt that in view of the controversial

nature of the subject the terms should be qualified.   The latent and bound amylases may be understood as those which are inactive, as evidenced by their inability to catalyze their respective characteristic reactions.   This inability may be due to either physical or chemical causes, according to prevailing controversial theories.   If the inability is due to physical causes, wherein the surface of the enzyme is thought to be mechanically isolated from the sphere of activity by effects of adsorption or by enveloping protein films, the enzyme may conveniently be designated as a "bound" enzyme.   If, on the other hand, the inactivity is due to chemical causes, wherein the enzyme is thought to be restrained by chemical inhibitory effects and rendered dormant, or the dormant enzyme is thought to be stimulated by chemical activatory effects and rendered active, the enzyme may be designated as a "latent" enzyme.   As will be evident in this paper, salt solutions liberate "bound" amylases from barley.

Since the presence and quantity of enzyme is manifested only by the extent of its activity, the bound or latent enzyme must be released from its inactive state and brought into a condition of activity in order that its presence may be detected and its quantity measured.   In order to effect this release, special methods of extraction must be employed.   The special methods used by various investigators in the extraction of the bound or latent amylase involve the use as extraction media of solutions of salts, peptone, proteolytic enzymes such as papain and pepsin, and such reducing substances as hydrogen sulphide and hydrocyanic acid.   Each investigator advances his own interpretation regarding the mechanism of the liberation of the inactive enzyme through the presence in the extraction medium of any of the specific substances mentioned.   The subject of this work, however, is the nature of the liberation of inactive amylases by only one of the specific substances mentioned, namely, salts.

Résumé of Literature

More than a decade before the importance of pH in relation to enzymic activity and the dual aspect of the diastatic enzymes were recognized, Ford and Guthrie (1908) made the important observation that the addition of salts, proteolytic enzymes, or certain other substances to barley meal increased the diastatic activity of the meal. They explained the increase in diastatic activity caused by the addition of salts by the assumption that the enzymes were protected from destruction which would otherwise occur in the absence of salts.   The protection of the enzymes was believed afforded by accompanying substances previously present, but put into solution by the added salts.

The observation of the increase in diastatic activity by Ford and Guthrie was confirmed in its essentials by a number of later investigators.

The work of Ford and Guthrie was reinvestigated and elaborated by Józsa and Gore (1932), who examined the increase of the saccharifying and particularly the liquefying activity in wheat flour, malt, and barley, caused by the presence of added salts and proteolytic enzymes in the extraction media. These authors believe that the presence of salts in the extraction medium brings about the observable increase in diastatic activity by increasing the solubility of the liquefying and saccharifying enzymes. Since the influence of the salt becomes less pronounced with increasing salt concentration in the extraction medium, this action was thought by the authors to be a purely physical one. This may be interpreted as indicating a bound condition of the enzymes.

Chrzaszcz and Janicki (1933) have developed an elaborate theory to explain the increase of amylase activity which is exhibited during barley germination or through the use of special extraction methods. They believe that a latent form of amylase exists in barley which is rendered active by substances formed during germination. According to these authors, there are two substances in germinating barley, an inhibitor and activator, called sisto-amylase and eleuto-amylase respectively. With progressive protein degradation, increasing quantities of eleuto substances are formed, leading to the stimulation of the latent enzyme. The sisto substance, it is believed, has no destructive action on the amylase but merely inactivates it through adsorption or by the formation of a complex association product. Both the sisto-amylase and the eleuto-amylase accumulate during germination of barley, the latter counteracting the former. According to these authors, the eleuto substances could be neutral salts like NaCl or could be protein degradation products like peptone. Hind (1938) points out that the above authors' "conception of the development of enzyme activity is not universally accepted and the present state of uncertainty in regard to the nature of diastase is made more apparent by the suggestion that its activity is determined not only by the presence of activators but, more particularly, by substances which inhibit its action." DePlanque (1936) in elaborating upon the sisto-eleuto system of Chrzaszcz and Janicki, compares the action of salts as eleuto substances to that of peptone. Thus when peptone is added to the malt or barley infusion there is an increase in soluble protein that is foreign to the system, while with the addition of salt there is an increase of soluble protein that is native to the system.

Following is the experimental work carried out for the purpose of extending the interpretations of certain investigators whose opinions have been referred to above.

Experimental

In view of the fact that the amylases of wheat flour are more rapidly and completely extracted by neutral salt solutions than by water alone, the possibility suggested itself to Blish, Sandstedt, and Kneen (1938) that the amylase is most highly concentrated in the globulin fraction, and Hopkins and Krause (1937) indicate that the inactive barley amylase is bound to the hordein fraction. Myrbäck and Myrbäck (1936) believe that the inactive amylase may be completely liberated from the protein, to which it is thought to be bound, by means of proteolysis. Bayliss (1925) states that the solubility of certain enzymes is closely related to the dispersive power of the solvent. In this connection, it may be thought that the greater the dispersive power of the solvent (dispersion medium) the greater will be the quantity of amylase colloidally dispersed from the particles of insoluble matter (proteins) to which they possibly are adsorbed or bound. If the bound amylase is adsorbed on proteins, it is possible that the dispersion of these proteins would bring about the liberation of a portion of the bound amylases. Gortner (1937) has shown that the degree of dispersion or peptization of proteins of cereals varies with the concentration of the salt solution and with the nature of the salt. The following experiments were designed to determine whether there may be a relationship between the peptization of proteins and liberation of bound amylase.

According to Dull and Swanson (unpublished data) the apparent maximum quantity of bound amylase is liberated by approximately $0.1 M$ salt solutions. Concentrations of salt greater than $0.1 M$ have a depressing effect on the diastatic activity. In order to measure the quantity of bound amylase liberated by salt solutions of concentration greater than $0.1 M$, a method must be employed that will avoid the error caused by the depressing effect of the salt. After considerable preliminary experimentation, a method was adopted similar to that used by Sandstedt, Blish, Mecham, and Bode (1937) in their investigations of residual amylase activity in wheat flour. By this method the salt contained in the extraction medium is washed out of the residue, upon termination of the extraction time, thereby eliminating the depressing effect of the salt. The amylase activity of the washed residue may subsequently be determined. The amount of bound amylase liberated may be computed as the difference in amylase activity in the residue of a barley sample extracted with distilled water

and that in the residue of a duplicate barley sample extracted with a salt solution.

The bound amylase liberated and the accompanying protein peptized by KCl solutions of 1%, 5%, 10%, and 20% concentrations, were determined on three 1938 American barley samples: (A) Wisconsin Pedigree 38, (B) Atlas, and (C) another Wisconsin Pedigree 38. The data are compiled in Table I. The experimental procedure is as follows:

To each of five 50-ml. capacity centrifuge tubes was added a one-gram portion of a finely ground barley sample, together with 25 ml. of extraction medium—distilled water, 1% KCl, 5% KCl, 10% KCl, and 20% KCl, respectively. The tubes and contents were kept at 20.0° C. and were shaken at 30-minute intervals during a four-hour extraction period, after which time the tubes were centrifuged, the supernatant liquid drawn off and filtered directly into a 500-ml. Kjeldahl flask. The residue, still contained in the centrifuge tube, was washed with 25-ml. portions of distilled water five times. The washing of the residue with distilled water is carried out for the purpose of completely removing the salt and of redispersing and removing the salt-coagulated proteins to effect also the removal of occluded or adsorbed amylases. After each addition of 25 ml. of wash, the contents were shaken, centrifuged, and the supernatant liquid filtered into the Kjeldahl flask. The collected filtrates in the respective Kjeldahl flasks served for the determination of the soluble protein-nitrogen, carried out in the usual manner, and calculated to milligrams of soluble nitrogen per 100 grams of sample on dry basis.

The residues in the tubes were quantitatively transferred to a 300-ml. volumetric flask with the aid of a minimum amount of ice-cold water. When each transfer was completed, the 300-ml. flask was immersed in an ice-water bath for the purpose of preventing diastatic autolysis, while the remaining transfers were being made and the necessary apparatus was being made ready to conduct the determination of diastatic activity in the residues. When the apparatus was in readiness, the 300-ml. flasks were quickly brought to 20.0° C. and 200 ml. of a 2% Lintner starch solution, buffered with sodium acetate-acetic acid buffer to a pH of 4.7, was added. The diastasis was permitted to proceed for exactly 30 minutes, after which time it was terminated by the addition of 10 ml. of $0.5N$ NaOH solution. The flasks were then made up with distilled water to capacity, and the maltose produced by diastasis was determined by the ferricyanide method of Laufer, Schwarz, and Laufer (1938), and calculated to grams of maltose produced per 100 grams of sample on dry basis.

The data, compiled in Table I, show that the extent of bound amylase liberated and the extent of protein-nitrogen peptized by salt solutions are almost parallel.   This may be interpreted as a strong indication that the bound amylases, released by salt solutions, are released as a result of peptization of the proteins to which they are bound.

TABLE I

THE RELATIONSHIP BETWEEN THE LIBERATION OF BOUND BARLEY AMYLASES AND THE PEPTIZATION OF PROTEINS AS EFFECTED BY KCl SOLUTIONS OF VARYING CONCENTRATION

	Extraction media				
	Dist. H_2O	1% KCl	5% KCl	10% KCl	20% KCl
A. 6-ROW WISCONSIN PED. 38 BARLEY (FREE ACTIVITY—325)					
Amylase activity [1] in residue	38	21	15	13	10
Bound amylase liberated (by difference) [2]	—	17	23	25	28
Protein-nitrogen peptized [3]	265	370	400	420	425
B. 6-ROW ATLAS BARLEY (FREE ACTIVITY—210)					
Amylase activity in residue	45	20	16	14	13
Bound amylase liberated (by difference)	—	25	29	31	32
Protein-nitrogen peptized	296	409	450	463	470
C. 6-ROW WISCONSIN PED. 38 BARLEY (FREE ACTIVITY—379)					
Amylase activity in residue	97	41	30	24	19
Bound amylase liberated (by difference)	—	56	67	73	78
Protein-nitrogen peptized	255	386	405	420	429

[1] Amylase activity calculated as g. of maltose per 100 g. dry sample.
[2] Bound amylase liberated calculated as the difference in amylase activity between that found in the residue extracted with distilled water and that found in the residue extracted with KCl solution.
[3] Protein-nitrogen peptized calculated as mg. of protein-nitrogen per 100 g. dry sample.

The data also show that there is still some residual amylase activity remaining after extraction by a 20% KCl solution, ranging from 10 to 19 g. of maltose per 100 g. of sample on dry basis.   The residual amylase after extraction with a 20% KCl solution is bound so securely that more effective liberating agents than neutral salts may be required for its release.   Such liberating agents as proteolytic enzymes or reducing substances may effect its release in addition to the release of still larger quantities of amylase that are bound beneath the exposed surface of the particles of insoluble portions of the barley residue.

Gortner (1937) has demonstrated that $0.5M$ concentrations of KF, KCl, KBr, and KI peptized increasing quantities of proteins from barley, respectively.   Consequently, to further test the hypothesis that the liberation of bound amylase is due to the accompanying peptization of the proteins to which the enzymes are thought to be adsorbed or bound, the following experiment was carried out.   The procedure is similar to that of the previous experiment, but uses as extraction media

0.5M solutions of KCl and KBr.   The data obtained on two barley samples, (A) 1938 Wisconsin Pedigree 38, and (B) 1938 O. A. C. 21, and on one kilned malt sample (C) of 1938 Manchuria barley, are given in Table II.   These data also show that there is a strong possi-

TABLE II

THE RELATIONSHIP BETWEEN THE LIBERATION OF BOUND AMYLASES AND THE PEPTIZATION OF PROTEINS AS EFFECTED BY 0.5M SOLUTIONS OF KCl AND KBr

	Extraction media		
	Dist. H_2O	0.5M KCl	0.5M KBr
A. 6-ROW WISCONSIN PED. 38 BARLEY (FREE ACTIVITY—320)			
Amylase activity in residue [1]	41	24	20
Bound amylase liberated (by difference) [1]	—	17	21
Protein-nitrogen peptized [1]	261	395	420
B. 6-ROW O. A. C. 21 BARLEY (FREE ACTIVITY—365)			
Amylase activity in residue	57	26	26
Bound amylase liberated (by difference)	—	31	31
Protein-nitrogen peptized	278	412	430
C. KILNED MALT OF 6-ROW MANCHURIA BARLEY (FREE ACTIVITY—505)			
Amylase activity in residue	55	30	25
Bound amylase liberated (by difference)	—	25	30
Protein-nitrogen peptized	660	832	880

[1] See Table I (footnotes).

bility that the extent of liberation of bound amylase by salt solutions is related to the extent of peptization of the proteins.   However, this relationship as presented in Table II is not strictly parallel.   Only by running a large number of these tests can the correlation be definitely established.   The amount of protein-nitrogen peptized by the 0.5M KBr was always greater than that peptized by 0.5M KCl, and to this extent was in agreement with the results of Gortner.

The slight lack of parallelism may be due to the possibility that protein particles are not alone in their ability to adsorb amylases, that the starch granules of barley and malt may play a role, however small, in the adsorption of the amylases.   This possibility is evident from the work of Holmbergh (1933), who experimented with alpha- and beta-amylase of malt, and the starches of rice, and has shown that the amylases can be adsorbed on starch granules.

Summary

Experimental evidence is presented that indicates that the bound amylase of barley, as released by salt solutions, is released as a result of the peptization of the proteins to which the enzyme is thought to be adsorbed or bound.

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CEREAL CHEMISTRY

PHYSICAL ASPECTS OF STARCH BEHAVIOR

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Until recently the various colloidal characteristics of a starch paste were attributed to the influence of specific component substances which presumably retained their identity under widely varying conditions. Due in large part to the association theory formulated by Meyer and Mark (1930), this has been supplanted by the concept of starch as one of the most complex of colloids, whose behavior is affected as much by physical environment as by actual chemical reactions.

With large molecules heavily loaded with hydrophilic groups, there arise associative forces which may approach or even exceed the stability of primary chemical linkage. The characteristic birefringence of the ungelatinized starch granule under polarized light is sufficient indication that the molecules have been deposited in some sort of orderly arrangement. Under such circumstances, the associative forces of adjacent molecules would be mutually satisfied, hydration tendencies would be at a minimum, and consequently the granule would be insoluble in cold water. If a water suspension of starch is slowly heated, the granules lose their birefringence before swelling becomes pronounced. This could be ascribed to partial hydration of the starch molecules, thereby disrupting their orderly arrangement. As heating is continued, the granules swell until they can no longer be readily distinguished under the microscope. Only then does the viscosity commence to rise. These changes are readily followed with the Brabender amylograph, whereby viscosity is charted against uniformly rising temperature. Figure 1 shows such a curve for raw corn starch, replotted on ordinary cross section paper. Samples were withdrawn at regular intervals and examined under the polarizing microscope.
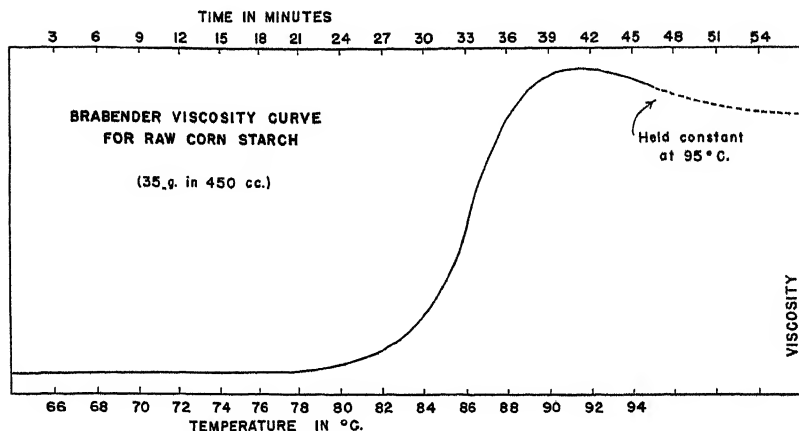
Figure 1.

Temperature	Microscopic Appearance
64° C.	No apparent effect.
66° C.	Slight swelling. Approximately 25% of granules have lost birefringence.
68° C.	Approximately 75% of granules have lost birefringence.
70° C.	Very few birefringent granules. Swollen approximately 2 ×.
72° C.	No birefringent granules.
74° C.	Swollen approximately 3 ×.
76° C.	Same.
78° C.	Slight additional swelling.
80° C.	Swollen approximately 4 ×. Granule outlines becoming vague.
82° C.	Same.
84° C.	Very vague.

However, much of the viscosity of a boiled starch paste must be attributed to swollen aggregates or to invisible fragments of granule structure, since such pastes can be radically thinned by autoclaving or by violent mechanical agitation. On prolonged autoclaving, the viscosity approaches a minimum which represents maximum dispersion of the starch. Such thinned pastes cannot be considered as true solutions, since the associative forces are still potentially active.

Water is not the only medium in which starch can be gelatinized. Liquid ammonia, liquid HCN, formamide, and formic acid are reputed to be excellent gelatinizing media. It will be noted that these are all liquids of high dielectric constant—in other words capable of dissociating the hydrophilic cohesion between starch molecules. Dissociation may likewise be effected by substances which are preferentially adsorbed on the hydroxyl groups of the starch, thus neutralizing intermolecular attraction. Sodium hydroxide solutions, above 1% concentration, will

gelatinize starch in the cold to give thick pastes or rubbery gels. This is merely a matter of adsorption, since the alkali can be completely removed by repeated precipitation with methanol. Saturated sodium hydroxide, on the contrary, will not gelatinize starch, possibly because it does not afford sufficient free water for hydration. Gels can also be obtained in solutions of certain salts, the familiar Hofmeister series, of which thiocyanates have the highest peptizing action. As in the case of sodium hydroxide, this gelation proceeds only as far as swelling, without any real disintegration of internal granule structure to produce a solution. The sodium hydroxide gel can be liquefied by heating, but this obviously involves chemical decomposition. However, if the peptizing ion carries a large inert residue, as in the case of the quaternary bases, then the solution state is more closely approached. For example, while starch is not affected by cold ammonium hydroxide, it readily dissolves in a cold aqueous solution of trimethyl benzyl ammonium hydroxide to give a limpid solution of low viscosity. Lieser and Ebert (1937) have observed a similar effect with cellulose, and they find that the peptizing activity rises with increasing molecular size of the quaternary base. They suggest that the quaternary ammonium ion acts as a wedge to pry apart the individual carbohydrate molecules. Morpholine, trimethylamine, and ethylene diamine have a similar action in hot solution, in these instances without apparent alkaline decomposition. Here it may be the hydrophilic amine group which associates with the starch by a process akin to hydration.

The solubility of starch can be raised by certain chemical modifications which might be mentioned at this point, though the mechanism of the reactions involved cannot as yet be satisfactorily explained. For example, when starch is dextrinized by heat, its solubility is markedly increased. There is some reason to believe that molecular water is eliminated to give an internal glucosan structure, and such products might be expected to show decreased associative tendencies. Even more obscure is the action of oxidizing agents. Treatment of raw starch with alkaline hypochlorite or peroxide increases solubility and decreases viscosity. Some oxidation of primary alcohol groups might be expected—in fact traces of uronic acids have been detected on hydrolysis of these starches—but this is scarcely sufficient in itself to explain the considerable change in character. With thin boiling starches produced by acid treatment, the manifest reaction is one of glucosidic hydrolysis, reducing the size of the starch molecule down from its highly associative colloidal dimensions. However, there is evidence of some type of linkage in starch which is even more susceptible toward acid hydrolysis than is the normal glucosidic bond. The solubility of certain starch ethers can be

best explained by the association theory. If an alkaline starch paste is treated with propylene oxide, the resulting hydroxyalkyl derivative yields clear aqueous solutions of high stability. Since the free hydroxyl content has not been affected, and since there is no possibility of glucosidic hydrolysis, the enhanced solubility can only be explained as a sort of steric hindrance, the substituent groups interfering with association of the starch molecules. The effect is even more pronounced with ethylated starch, where the free hydroxyl content has been reduced.

When granule structure is disintegrated by prolonged dry-grinding in a ball mill (Alsberg and Perry, 1924), the viscosity is markedly reduced. Unfortunately, the so-called alkali lability increases (Taylor, Fletcher, and Adams, 1935), indicative of some sort of hydrolytic scission. In this connection, it might be remarked that the efficiency of granule disintegration is greatly improved by wet-grinding an alcohol slurry of the starch. But even by this method, the alkali lability rises somewhat.

In a certain sense, the selective action of certain solvents in removing fatty material from the cereal starches is indicative of the hydrophilic bonding within the granule (Schoch, 1938). Only those fat solvents of a hydrophilic character can penetrate into the granule. If bone-dry starch is confined over various volatile liquids, vapor adsorption is dependent on the hydrophilic nature of the liquid. In discussing the extraction of chlorophyll from green leaf tissue, Bancroft and Rutzler (1938) suggest that only those solvents which are preferentially adsorbed by the cellulosic leaf tissue can desorb the chlorophyll, with consequent removal of the latter. A similar mechanism should apply to the fatty acids and phospholipids in the various cereal starches.

If starch is first peptized by alkali, or by the quaternary bases, or by salts of the Hofmeister series, then the peptizing agent removed (as by alcohol precipitation, or electrodialysis, or neutralization with acid), the starch is thrown out as a mushy paste. With trimethylamine, a similar phenomenon results if the volatile base is distilled off. All such tendencies of starch to revert to less soluble forms are given the broad designation of "retrogradation," paralleling the denaturation of proteins. Examples are plentiful. If an autoclaved starch paste—representing the maximum degree of dispersion which can be achieved without hydrolytic degradation—is allowed to stand at room temperature, there is a gradual flocculation of insoluble material. To a limited extent, this can be re-dispersed by further autoclaving. Retrogradation proceeds more rapidly, and is more complete at low temperatures. If a starch paste is frozen, then thawed, it gives a spongy fibrous mass representing the total original starch (Woodruff and MacMasters, 1938). This may be

partially dissolved by autoclaving, but each subsequent freezing renders it more resistant toward solution. If we conceive of freezing as dehydration by ice formation, then association between starch molecules could reassert itself.

The speed of retrogradation is dependent on the concentration of the starch paste or solution. Thus an autoclaved 1% starch solution will remain fairly clear for several days, while a 2% solution will flocculate over night. Solutions of 3% are highly unstable, and may gel out spontaneously in an Ostwald pipette during a viscosity determination. If an autoclaved 1% solution is evaporated *in vacuo* on the steam bath, it will suddenly revert to an insoluble mush when the concentration reaches 3% — 5%. Addition of water at this point does not effect re-solution.

Another instance of retrogradation is the skinning of boiled starch pastes through surface evaporation. The significant point is that such skins are very difficult, if not impossible, to redisperse.

Alcohol precipitation of starch from pastes or solutions may be considered as involving retrogradation. In the first place, such precipitation is in marked contrast to that of crystalloids where there is a definite solubility at each concentration of alcohol and water. If methanol is slowly added to an autoclaved starch solution, there is no pronounced change until the alcohol concentration reaches approximately 40%, when the solution becomes opaque. When the concentration reaches 45%, the starch is completely flocculated. Attempts to separate possible starch components by such fractional precipitation have not been successful, because of this very narrow range. The concentration of various precipitants necessary to initiate flocculation is significantly in line with the dielectric constant:

Acetone or diacetone	32%
Methyl or ethyl alcohol	38%–40%
Methyl cellosolve or dioxan	53%–56%
Ethylene glycol or glycerol	above 70%

Starch which has been precipitated by alcohol cannot be redissolved to give anything approaching its former degree of dispersion, even by autoclaving. Such retrograded starch can be dissolved in alkali, though with some difficulty. This might be anticipated, since alkali-peptized starch does not retrograde on freezing or on standing, nor does it exhibit the skinning effect observed with starch pastes.

Retrogradation is favored by low pH. Solutions of starch which have been buffered to various pH levels and allowed to stand will show a progressive increase in precipitation as the pH is lowered. Below a pH of 3, the amount of precipitated material diminishes, due to hydrolysis of the starch.
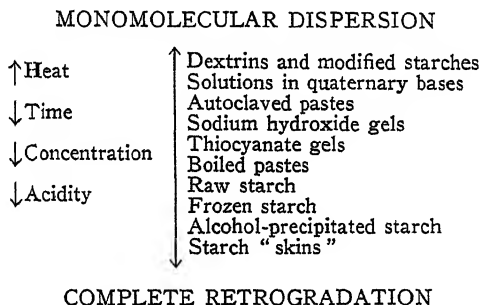
Certain polar substances containing a heavy hydrophobic loading cause precipitation of starch, though in somewhat different fashion. Cyclohexanol is particularly effective in this respect, precipitating the starch completely and immediately from hot solution. This differs from normal retrogradation in that the starch redissolves when the cyclohexanol is removed by steam distillation. This action might be visualized as adsorption of the cyclohexanol through association of its hydroxyl group with those of the starch, thus coating the latter with a waterproof film of oriented cyclohexane radicals and preventing hydration. A number of wetting agents have a similar action, as well as fatty acids and higher alcohols, soaps, sulfonated oils, etc. Familiar to the starch chemist is the fact that a tapioca starch paste—normally slimy and transparent in character—is converted into a " short " opaque paste by the addition of a small amount of sulfonated castor oil. The slimy nature of tapioca starch can only be attributed to a high degree of molecular association, approaching that of a gel. If a dilute tapioca paste is rapidly stirred to impart a swirling motion, this is quickly damped, then a reverse motion is set up, and the paste may wind and unwind for several seconds. This would indicate a skeleton of molecular forces operating throughout the paste. Now if a sulfonated oil is added, the paste loses most of this back-lash effect, behaving more as if it were composed of discrete particles whose only relation to one another was one of mechanical friction. Some of the physical differences between the tuber and cereal starches can be ascribed to the presence of polar fatty acids in the latter. Cornstarch pastes are shorter, less slimy, and more opaque than those from tapioca starch. However, if the fatty acids are removed from the cereal starches by Soxhlet extraction with methanol, then the resulting starch yields pastes which are much more transparent and slimy. As another example, a 5% paste of raw rice starch must stand for a day or more to give a weak gel, while a similar paste of de-fatted rice starch sets up to a strong gel immediately on cooling to room temperature.

The action of borax to produce rubbery gels with solutions of starch or vegetable gums is well known. Electrometric titration curves indicate that the ionization of boric acid is increased by the addition of starch or dextrin. So the gelling action might well be related to the complex formation between borate ion and such polyhydroxylated substances as glycerol, glucose, and mannitol. If borate acted as a bridge between adjacent starch molecules, the anticipated effect would be an increase in viscosity and gelling characteristics.

Those modified starches whose solubility has been enhanced by such means as dextrinization, or oxidation, or ethylation, show little tendency

to retrograde. Solutions are stable over extended periods of time, do not readily skin over, or if a skin does form it can be readily dissolved. They are less sensitive toward precipitation by polar substances, and when flocculated by excess alcohol, can be readily redissolved in water.

All this adds up to a fairly reasonable and consistent picture. The affinity of starch for water is opposed by the more powerful association forces operating between the starch molecules. Consequently, hydration proceeds only far enough to give colloidal gels, or with extreme difficulty to give colloidal solutions. These are meta-stable, and require little provocation to revert to the more stable insoluble state. More complete dissociation of the starch molecules can only be accomplished by agents possessing greater ability than water to satisfy the intermolecular forces. The various degrees of starch dispersion may be crudely visualized as a sort of spectrum:

MONOMOLECULAR DISPERSION

↑Heat	Dextrins and modified starches
	Solutions in quaternary bases
↓Time	Autoclaved pastes
	Sodium hydroxide gels
↓Concentration	Thiocyanate gels
	Boiled pastes
↓Acidity	Raw starch
	Frozen starch
	Alcohol-precipitated starch
	Starch "skins"

COMPLETE RETROGRADATION

Few investigators have ever agreed on the ratio of soluble and insoluble amyloses in starch, estimates running the entire gamut from 100% amylose to 100% amylopectin, depending on the mode of isolation. With such a meta-stable substance as starch, it is obvious that the amount of any such fraction would be governed by the degree of dispergation of the granule, the amount of retrogradation which occurred, the effect of chemical degradation (as by acid hydrolysis), and a host of similar factors to which little heed has been given. As a result of this confusion, there has lately been a trend to disregard such possible components and to consider starch as a single chemical entity.

But recent evidence may reopen this question, since fractions have been isolated from starch which are markedly different, not only in physical characteristics but likewise in chemical reactivity. When an autoclaved starch solution is treated with excess butyl or amyl alcohol, there occurs a slow precipitation at the alcohol-water interface. This material is semi-crystalline in appearance, minute spheroids or needles,

quite different from the amorphous floc of retrograded or methanol-precipitated starch. The supercentrifuge is the only satisfactory means of isolating this product, to give a creamy white deposit. If this is dehydrated with alcohol, it becomes completely insoluble. However, if removed from the centrifuge and immediately treated with boiling water, it readily dissolves to a clear thin syrup, even at concentrations as high as 20% solids. Such a solution shows greatly exaggerated retrograduation tendencies. If allowed to cool even slightly, it sets to a rubbery translucent gel which cannot again be liquefied by heating. It can be purified by reprecipitation with butanol, consistently amounting to 21%–23% of the original starch. The remainder of the starch can be precipitated from the centrifuged solution with methanol. In contrast, this material is readily soluble in water to give clear stable syrups. But the most significant difference lies in their respective alkali labilities, the insoluble fraction being much more labile than is the parent raw starch, while the soluble fraction is correspondingly more alkali-stable. Such behavior can only be ascribed to variation in chemical configuration. This study is being continued, in an effort to identify the structural differences.

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A METHOD FOR THE DETERMINATION OF REDUCING MATTER IN FLOUR

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(Read at the Annual Meeting, May 1940)

In connection with our work on the effects of oxidation in doughs, it is of great interest to know the relative quantities of reducing matter present in different flours, since it is known that such substances stimulate protease activity, and we have shown that additions of reducing factors such as those present in wheat germ produce great differences in baking quality.

An examination of some of the commonly used books on food analysis revealed no detailed method for the determination of reducing matter in products such as flour and cereals. Brief outlines of two such methods were found in a paper by Sullivan, Howe, and Schmalz (1937). By one method, the proteins are removed with sodium tungstate and sulfuric acid; then a titration is made with KIO_3 in KI and thiosulfate, using a potentiometer. By the other method, an iodine titration is used after removal of the proteins by use of sulfosalicylic acid.

Woodward and Fry (1932) found that autoxidation with loss of reducing matter took place in tungstic acid filtrates even at pH 3.0, and that with trichloracetic acid the recovery of added glutathione was incomplete, but that with sulfosalicylic acid there were both good recovery and no autoxidation.

Preparation of Concentrated Flour Extracts

The availability of the Waring Blendor, an efficient, high-speed stirrer, made it possible to use flour extracts more concentrated than those used by Sullivan in the tungstic acid method.

Table 1 shows the variations in iodine uptake between extracts prepared as in the tungstate procedure and more concentrated extracts, prepared with the aid of the Waring Blendor.

These results showed: (1) that the use of the Waring was as good as hand shaking in the tungstate procedure, (2) that the Waring gave considerably more reducing matter in the more concentrated extract as compared to hand shaking, and (3) that the value for reducing matter in the more concentrated extract was very much greater than in the Sullivan procedure.

TABLE I

AMOUNTS OF IODINE SOLUTION TAKEN UP BY FLOUR EXTRACTS OF DIFFERENT
CONCENTRATIONS, PREPARED BY MANUAL SHAKING COMPARED TO
MECHANICAL STIRRING AND CENTRIFUGING

	Iodine solution used by 100 cc. of flour extract (cc. of 0.010 N) [1]
One part flour, 5 parts water:	
Shaken by hand (tungstate procedure)	1.05
Stirred in Waring for 1 minute	1.1
One part flour, 3 parts water:	
Shaken by hand, centrifuged	1.5
Stirred in Waring, centrifuged	1.9

[1] The titrations were made by adding 10.0 cc. of the iodine solution and titrating back with thiosulfate solution, using starch indicator.

Effect of pH on the Amount of Iodine Taken Up by Flour Extracts

That the control of pH is of prime importance was indicated by tests in which a plain water extract of a low-grade flour took up six times as much iodine as an equal quantity of the same extract in which tungstic acid was used.

The effect of pH on the amounts of iodine taken up by extracts of patent and low-grade flours are shown in Table II and Figure 1. The flour extracts were prepared by stirring 1:3 flour-in-water suspensions in the Waring Blendor, centrifuging, boiling the liquids, and filtering off the coagulated protein; the filtrates were made up to volume, and 50-cc. portions were titrated after additions of acid to give increasingly lower pH values.

It is evident that there is a marked decrease in the quantity of iodine taken up by the reducing matter in the extracts with decreasing pH, and

TABLE II

EFFECT OF pH ON AMOUNT OF IODINE TAKEN UP BY EXTRACTS
OF PATENT AND LOW-GRADE FLOURS

Patent			Low grade	
pH	Iodine used		pH	Iodine used
	cc.			cc.
5.92	2.1		6.1	4.4
4.72	1.2		5.42	3.2
4.30	0.9		4.9	2.6
3.87	0.8		4.2	1.7
3.55	0.6		3.18	1.2
3.0	0.6		2.65	1.1
2.7	0.6		2.37	1.05
2.3	0.6		1.9	0.9
1.9	0.55			
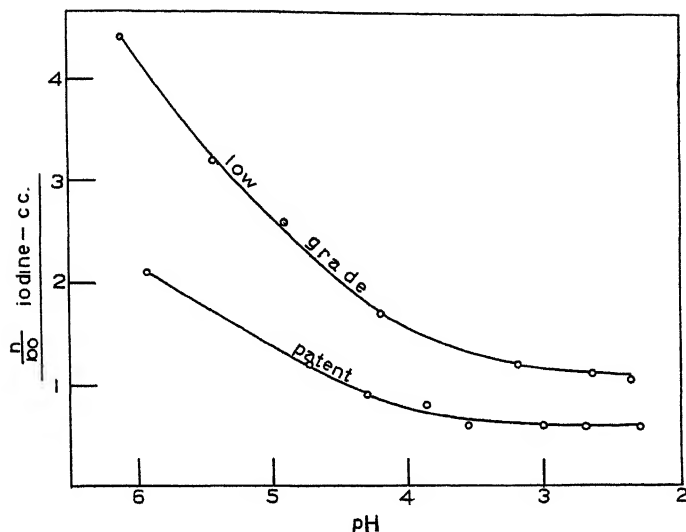
Fig. 1. Effects of increasing acidity on the amount of iodine taken up by flour extract.

that the values for each flour tend to become constant below pH 3.0.   It is therefore evident that for comparable results, titration should be made in highly acidified extracts.

Because of the high buffer value of flour, and the great differences in buffer value between patent and low-grade flour, it was indicated that the most desirable procedure was probably the addition of a large enough excess of acid to give a sufficiently low pH even with the most highly buffered flours.

Factors in Loss of Reducing Matter through Oxidation

That appreciable amounts of reducing matter were lost, probably through oxidation, was indicated by experiments in which (1) variations were made in time of stirring in the presence of air, (2) air in the flour was replaced by nitrogen before stirring, and (3) titrations were made at different times after starting the filtration of the liquid following centrifuging.   The results of these experiments are shown in Table III.

The figures in Table III indicate (1) a slight increase in reducing matter due to removal of air from the flour, (2) a great loss in reducing matter due to long stirring while the suspension is exposed to air, and (3) slight loss in reducing matter due to standing exposed to air during and after filtration.

To avoid exposure to air during the stirring of the suspension, the edge of the Waring container was ground smooth so that a piece of flat

TABLE III

EFFECTS OF AIR IN THE FLOUR, THE TIME OF STIRRING OF THE SUSPENSION, AND THE TIME OF TITRATION AFTER THE START OF FILTRATION, ON THE AMOUNTS OF IODINE TAKEN UP BY FLOUR EXTRACTS

	Iodine used (cc. of 0.010 N)
Air in the flour:	
Flour stirred in an atmosphere of nitrogen to remove air ..	1.85
Flour not treated	1.70
Time of stirring in Waring:	
1 minute..	3.2
5 minutes..	1.9
Time of titration after start of filtration:	
15 minutes..	3.50
75 minutes....	3.35
135 minutes.......	3.25

plate glass would fit tightly over it; it was then completely filled with the suspension so as to exclude as much air as possible.[1]

Time of Standing after Stirring

Tests in which the suspension was allowed to stand for varying time intervals before centrifuging, gave results as shown in Table IV.

TABLE IV

AMOUNT OF IODINE TAKEN UP BY FLOUR EXTRACTS AS AFFECTED BY THE TIME OF STANDING AFTER STIRRING AND BEFORE CENTRIFUGING THE SUSPENSION

Time	Iodine used (cc. of 0.010 N)
None	2.7
½ hr.	2.9
1 hr.	3.3
2 hrs.	3.3

The results showed that the suspension should stand about one hour before being centrifuged, to obtain close to maximum extraction.

Time between the Addition of the Iodine Solution and the Back Titration with Thiosulfate

It was found that the longer the extracts stood after the addition of iodine and before the back titration with thiosulfate, the greater was the amount of iodine taken up by the extract. Typical examples of this are shown in Table V.

TABLE V

EFFECT OF TIME ON THE AMOUNT OF IODINE TAKEN UP BY FLOUR EXTRACTS AND
SOLUTIONS OF GLUTATHIONE

	Iodine used (cc. of 0.010N)
Flour extracts:	
Titrated immediately	2.2
Titrated after standing 2 hrs. with added iodine solution	3.5
Solutions of glutathione:	
Glutathione, 10 mg.—titrated immediately	3.25
Glutathione, 10 mg.—titrated after standing 1½ hrs. with added iodine solution	3.9

It is therefore evident that the back titration with thiosulfate should be made as soon as possible after the addition of the iodine in order to avoid error. That the value after immediate titration is correct is apparent from the glutathione titration; multiplying 3.25 × 3.07—which is the mg. equivalent to 1 cc. of 0.010N iodine solution—the result, 9.98, is equal to the 10 mg. of glutathione actually used in the titration.

Use of Iodine Solution Compared to Potassium Iodate

In making titrations with standard KIO_3 and an excess of KI, the values obtained were found to be much lower as compared to iodine solution of the same normality. This was very puzzling at first; it was later found, however, that the iodate readings varied in part as the amount of KI used. After equalizing the amounts of KI used, the values for iodate and iodine solution were found to agree.

Table VI shows the differences between iodine solution and KIO_3 of the same normality, as affected by the amounts of KI used with the iodate. Figure 2 shows the effects of varying amounts of KI on the amount of iodine taken up by the wheat-germ extract.

TABLE VI

EFFECTS OF VARYING AMOUNTS OF KI USED IN TITRATIONS OF WHEAT-GERM
EXTRACTS WITH IODINE SOLUTION AND KIO_3 OF THE SAME NORMALITY

Standard solution:	Amount of KI in 10 cc. of the solution:	Iodine used by wheat-germ extract equivalent to 1.67 g. germ (cc. of 0.010N)
Iodine, 0.010N	0.24 g.	2.15
KIO_3, 0.010N	Large excess	1.3
KIO_3, 0.010N	0.25 g.	2.1
Increasing amounts of KI with 10 cc. of 0.010N KIO_3:		
KI (g.)		
0.2		2.15
1.0		1.8
2.0		1.6
4.0		1.45

Fig. 2. Effect of increasing amounts of KI on the amount of iodine taken up by wheat- germ extract.

It is evident from these results that the use of large excesses of KI must be avoided in titrations with flour extracts. (The use of large excesses of KI made no difference in titrations with sodium thiosulfate.)

Use of Sulfosalicylic Acid

After finding that 6 g. of sulfosalicylic acid per 100 g. of flour would produce satisfactory precipitation of the proteins, it was compared to tungstic acid, and to boiling and filtration, to note which was best suited for the purpose of protein removal. It was found that boiling and filtration did not remove all the proteins, as indicated by further precipitation after addition of sulfosalicylic acid to such filtrates.

It was also found that the rate of filtration was better for sulfosalicylic acid than for tungstic acid, and the former also seemed to give a slightly better end point in the titration.

Because of these advantages, as well as the reduced autoxidation found by Woodward and Fry (1932), it was decided to use sulfosalicylic acid in preference to the tungstic acid procedure.

Effects of NaCl

Efforts to obtain a clearer extract by using NaCl in conjunction with sulfosalicylic acid were not very successful, till it was observed that clear extracts were obtained from doughs containing about 2% NaCl. It was then found that if the NaCl is stirred into the suspension with

the flour, and the sulfosalicylic acid is added after allowing the suspension to stand for some time, fairly clear extracts are obtained. (In the earlier experiments, the NaCl and sulfosalicylic had been added at the same time.)

Method for Reducing Matter in Flour

The method for reducing matter in flour (or dough) as finally adopted, was as follows:

108 g. flour, 2 g. NaCl, and 314 cc. water (these quantities were found to be just enough to exactly fill a small Waring container) are stirred for 60 seconds in the Waring Blendor, after the flour is first stirred dry in an atmosphere of nitrogen. The suspension is allowed to stand in stoppered containers for 45 minutes at 30°C. and 10 cc. of a 60% solution of sulfosalicylic acid is then added to the suspension, which—after slight shaking by hand and standing at 30°C. for another 15 minutes—is centrifuged in stoppered glass centrifuge bottles. The centrifuged liquid is filtered through folded paper, and 100 cc. of the filtrate titrated back with $0.010N$ thiosulfate immediately after adding 10.0 cc. of $0.010N$ iodine solution. Two cc. of 1% starch solution is used as indicator.

Recovery of Added Glutathione

That this procedure probably gives fairly accurate values for reducing matter in flour was indicated by tests in which glutathione was added to flour suspensions. The results of one test are shown in Table VII.

TABLE VII

TITRATION OF GLUTATHIONE ADDED TO FLOUR SUSPENSION
20 mg. glutathione added

	Iodine used by 100 cc. of flour extract (cc. of $0.010N$)
Flour extract, control	1.95
Flour extract from suspension with added glutathione	3.70

Calculation:
    Observed difference due to added glutathione, $3.70 - 1.95 = 1.75$.
    20 mg. glutathione in water solution took up 6.45 cc. of $0.010N$ iodine.
    Calculated value $\frac{100}{338} \times 6.45 = 1.92$.

    (338 cc. is total volume of liquid in the suspension, including flour moisture.)

    $\frac{\text{Observed diff.}}{\text{Calculated diff.}} = \frac{1.75}{1.92} = 91.5\%$ of added glutathione present in the flour extract.

The presence of more than 90% of the added glutathione, as shown by the titration, indicates a fair degree of accuracy, considering the difficulties involved in the procedure.

Reducing Matter in Flours from Northwestern and Texas Wheat

Using the sulfosalicylic acid and NaCl method as described, the relative values for reducing matter in different grades of flour were found to be those listed in Table VIII.

TABLE VIII

REDUCING MATTER IN DIFFERENT FLOURS

Flours from Northwestern wheat	Iodine used (cc.—0.010N)	Flours from Texas wheat	Iodine used (cc.—0.010N)
Patent	0.9	Patent (80%)	0.9
Straight	1.2	100%	1.1
Clear	1.5	Clear (15%)	1.4
Low grade	3.5	Low grade (5%)	1.9

The values in Table VIII indicate differences in the amounts of reducing matter in different kinds and grades of flour; the lower grades contained the greater amounts of reducing matter, and the flours from the Northwestern wheat showed more reducing matter than the Texas flours; the Northwestern low grade contained by far the greatest amount of reducing matter.

Summary

A study was made of some factors involved in the measurement of reducing matter in flour by use of an iodine titration method.

Concentrated flour extracts were prepared by stirring 1:3 flour-in-water suspensions in the Waring Blendor (an efficient high-speed stirrer) and centrifuging the suspensions.

It was found necessary to use highly acidified flour extracts in order to obtain constant values for the amounts of iodine taken up by the extracts.

Oxidation, due to contact of the flour suspensions or extracts with air, was found to cause appreciable loss of reducing matter.

A method based on the use of sulfosalicylic acid and NaCl for protein removal, the use of the Waring Blendor, and the exclusion of air, is described.

The method was found to be fairly accurate, as indicated by the titratable values in terms of glutathione added to the flour suspensions.

Significant differences in the amounts of reducing matter in different flours were found by use of this method.

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THE USE OF DIELECTRIC MEASUREMENTS TO DETERMINE THE MOISTURE CONTENT OF POWDERY SUBSTANCES. II

V. B. Yevstigneyev [1]

In a previous article [2] were presented the results of experiments on the use of dielectric measurements to determine the moisture content of powdery substances. These results promised great possibilities of developing a perfectly satisfactory method suitable for practical application.

In order to avoid discrepancies in the results due to nonuniform grinding and density of packing of the substance in the condenser we applied a method of reducing the results to identical conditions by recalculating the experimental data according to a formula.

The best results, particularly for powdered biscuits, were obtained with the use of formula No. 1 deduced by the author on the basis of experimental data and Lichtenecker's logarithmic rule of mixtures:

$$\log E_1 = \frac{a_1^n \log E_2}{a_2^n}, \tag{1}$$

where E_1 is the dielectric constant of the dielectric formed by the substance with which the condenser is filled up to a certain level (that is, a mixture of the substance and air) when the weight of the substance is a_1; E_2 is the dielectric constant of the same substance when the weight

used to fill the condenser up to the same level is a_2; and n is an exponent $= 2/3 = 0.6667$.

If the dielectric constant is known for any given weight of the substance in the condenser, this formula may be used to determine the dielectric constant for any other weight of substance in the condenser. (It is to be understood that the condenser is always filled to the same volume.)

In the present work we give more detailed data obtained during verification of the method developed, together with further methodological development of this means of determining the moisture content of powdery substances from their dielectric properties. We worked with the same powdery substances as before, namely powdered biscuit, flour, and granulated sugar. However, attention was mostly directed to work on biscuit since in the previous work the most reliable results had been attained with this substance.

Determination of the Curve of the Dielectric Properties of Biscuit as a Function of Moisture Content

The problem of determining the curve showing the dependence of the dielectric properties on the moisture content should be studied with the greatest care, since the accuracy of all the subsequent work depends primarily on the correctness with which this is carried out. Attention must also be directed to the choice of material for study and to the means of obtaining specimens of different moisture content.

If we have to deal with a powdery substance with approximately identical grain size and of identical chemical composition in which only the quantity of moisture varies, then the choice of material presents no difficulties. In this case it is possible to use any specimens, attention being directed only to the selection of samples covering the desired range of moisture content.

For most of the products encountered in food industries the problem is not quite so simple. For instance, if we have to deal with meal products we find that the term " biscuit " covers a large number of products which differ greatly from one another in their chemical composition and physical properties. This is a result of using different recipes, different qualities and types of flour, of difference in the quantity of fats, different admixtures, etc.

Even with products the composition of which does not vary so greatly and which depend considerably less on human choice, such as grain, flour and tea, the difference between various kinds may be very considerable. In these cases the chemical composition and physical

properties of a single substance may vary appreciably, depending on the conditions of growth, weather, soil, climate, etc.

Differences of this kind are important because the results of measurements of the dielectric properties depend to a considerable degree on the chemical composition of the dry residue; moreover the influence of this is evident not only through changes in the quantity ratio of substances with different dielectric constants, but also through changes in the dielectric losses, in certain cases depending on the state of the water in the substance.

The second case is usually encountered on change in the ash content of the food product and the third on change in the quantities of component parts associated with water, for instance colloids and, particularly in the case of flour and dough, the quantity of gluten. Changes in chemical composition influence the results to a greater or less extent, depending on their size and kind, and are of an individual character for each product. It is therefore necessary to exercise great care and to take into account all the circumstances in choosing the material with which to determine the calibration curve for a given product. It is of course best, in view of these considerations, to specialize the calibration curve as far as possible, and to determine it for only one type of food product with the most constant composition of the dry substance.

In cases where this procedure is altogether impossible it is necessary to approximate it as closely as possible and to determine the curve for, say, a group of different varieties of the one product. For instance our experience shows that the differences between the physical-chemical properties of various kinds of sugar biscuits manufactured by the Moscow factories are not serious enough to prevent the consideration of " sugar biscuits " as an approximately uniform group. It is of course obvious that the moisture content of biscuits is such that comparatively small changes in the ash content should have no appreciable influence on the value of the losses. Differences in the chemical composition of the dry residue of the biscuit may be of inconsiderable influence owing to the small difference between the dielectric constants of the component parts. In these types of biscuits the gluten effect is largely overcome by the large quantities of sugar and fats so that changes in gluten content do not play a basic part. Finally, differences in structure are largely eliminated by the grinding process. As a result of these circumstances we were able to draw up a single curve for sugar biscuits, without subdividing the group according to the name of the biscuit and the place of manufacture.

The problem of obtaining specimens with different moisture contents necessary for the determination of the curve must be approached with care. There are two conceivable ways of doing this. First we

may select one specimen of the product which is most typical of the entire group in composition and alter its moisture content artificially. With this method it is easily possible to obtain a smooth curve with the defect, however, that it does not always express sufficiently well the relationship for the entire group.   The method of varying the moisture content of the initial specimen is of great importance, at any rate in the case of powders.   The least satisfactory method is to add different quantities of distilled water to a weighed amount of the initial substance. In this case, even if the subsequent mixing is very carefully carried out there is danger of nonuniform distribution of the water throughout the mass of the product.   This in itself causes inaccurate dielectric measurements, since the results of these depend not only on the quantity of the substance but also on its spatial arrangement.   In the case of ground biscuits the best results may be obtained by keeping the specimen over water in a closed space and mixing it constantly.   This requires considerably more time, and again does not immediately lead to the requisite uniform distribution of moisture content.   The specimens of increased moisture content which are obtained must be mixed very carefully and allowed to stand in a hermetically sealed vessel for two to three days before commencing the measurements.   It is obvious that hygroscopic moisture cannot immediately penetrate quite uniformly throughout the entire thickness of the biscuit particles.   Favorable conditions for obtaining a uniform distribution of water are afforded when one uses not simply hot water but certain saturated solutions of salts, which ensure a definite relative humidity of the air.   The absorption of moisture will then proceed until the establishment of equilibrium between the vapor pressures above the surface of the biscuit and above the solution used, which occurs only when the moisture is uniformly distributed throughout the biscuit.   However, the application of these solutions is more complicated and the total time required for complete absorption is not less but is actually considerably greater than with absorption over water.

The method of obtaining the curve by artificial variation of the moisture content is quite satisfactory and is even preferable to other methods for a preliminary investigation of the question as to whether there is a relationship between the moisture content of a substance and its dielectric properties.   If one and the same specimen of the product is used for all the measurements, the influence of all other factors except the moisture content is excluded.   However, in our opinion a more practical way of obtaining the curve is to take specimens of biscuit at different times from the factory and the shops with the object of obtaining products of different moisture content.   A much greater number of specimens for investigation is needed with this method since the points, when plotted, do not usually lie on a smooth curve, as is almost always the

case when the moisture content of a single specimen is altered artificially. This effect, as previously noted, is primarily due to differences in the composition, structure, and similar properties of the specimens of different origin and composition.

The curve which is determined in this case nevertheless expresses the relationship between the dielectric constant and the moisture content. in a manner which is much more likely to be correct for all types of sugar biscuit than when a single specimen is moistened artificially. This is because such a curve will characterize a certain sort of biscuit with average properties for the given group, and it is clear that the greater the quantity and variation in quality of the specimens investigated the more the curve obtained will be characteristic of the entire group, even though it is not altogether accurate for each individual case. The amount of scattering of the experimental points shows the validity or the inadvisability of uniting the chosen products into a single group. In choosing specimens for this method it is therefore necessary that the requisite variety of types of biscuit as well as different moisture contents be obtained.

The methods of preparing the specimens for analysis, of grinding them into a powder, of determining their moisture content, and of taking into account the density of packing may be varied at will with either of the methods described, but once these have been chosen for a given series of measurements they should be maintained unaltered until the series is completed.

Here it is necessary to mention the importance of the temperature at which the measurements are carried out. If the proper correction is not determined then the temperature must be allowed to vary as little as possible, say to less than $\pm 2°C.$, in order to avoid large errors. The results of experiments confirming this assertion will be shown later.

Experimental Procedure

In experiments which were carried out with the object of developing and verifying the method of determining the moisture content of biscuits from the dielectric constant, measurements were made at a temperature of $20 \pm 2°C.$, using 25 specimens of biscuits obtained from the factory and from shops.

These specimens were ground with a mortar and pestle (but not too finely, in order to avoid unnecessary difficulties in the work) and were mixed in a vessel with a ground-glass stopper. The moisture content of the specimens was determined by the oven drying method, keeping them at a temperature of 130° C. for 40 minutes, which is taken as a standard in the manufacture of biscuits.

The ground specimen of biscuit was poured into the condenser up to a certain mark.[3]   The method of filling the condenser with powder is not of great consequence; however the more uniformly and evenly the initial filling is carried out the better the agreement between repeated measurements.   In our experiments we usually poured the powder into the condenser through a wide-mouthed funnel right up to the top of the condenser and then packed it down by gentle tapping and finally filled the condenser up to the mark with powder, packing it down in the same manner.   Coincidence between the level of pouring and the mark on the condenser was determined by the eye.   When the diameter of the upper part of the vessel is small only a slight error will occur.   The filled condenser was immediately placed in the instrument and the capacitance reading was made, using the "Dielkometer" as described previously.   Following this, the condenser was weighed to the nearest 0.1 g., to obtain the weight of the sample.

At the beginning of the experiment a control measurement was made with the condenser empty.   With identical experimental conditions the reading should always be the same.   In the event of small deviations due to differences in temperature or to fall in the accumulator voltage, a correction may be introduced.

We used this method of measurement for the preliminary and for the first verificative investigations; all the other measurements were carried out by a second method in which the fine condenser only was taken as the basis.   For this method, at the beginning of a measurement the fine condenser scale was set at 90, the empty measuring condenser was placed in the circuit, and a reading was found with the rough tuning. This was taken as the zero position and the tuning of the rough condenser was then left unaltered.   After the powder to be investigated had been poured up to the mark the reading was found by adjusting the scale of the fine condenser and this reading was taken as the value of the measurement.

The reading obtained by one method or the other is of purely arbitrary magnitude which depends both on the individual peculiarities of the instrument and on the capacities of the measuring and standard condenser.   These readings may be used directly only to construct a curve either for powders which always fill the condenser in the same manner or for rough orientational measurements, since the dielectric constant of a powdery dielectric depends on the ratio of the volume occupied by the actual particles of powder to the volume of the spaces between them, that is, on the packing density.

In order to avoid the influence of packing density it must either be equalized or taken into account in some way.   Generally speaking it is

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[3] For a description of the measuring condenser see Cereal Chem. 16: 342.

possible to find experimentally the dependence of the instrument readings on the density of packing, characterizing the latter for instance by the weight of substance which is in the condenser. However in order to include all the possible cases of sample weight variation for different moisture contents in sufficient number for the determination of curves it is necessary to carry out a very large number of measurements. It is more convenient to recalculate the values and to relate them to a certain weight, as we did, since a much smaller number of specimens are required. The formula used for these calculations was given in the early part of the present paper. The weight to which the measurements are to be related may be chosen arbitrarily, but it is of course necessary to choose a suitable value and to avoid referring the data to a weight which cannot be put into the condenser.

We usually referred the results of our measurements to 100 g. simply because the condenser which we used holds approximately this quantity of biscuit. For reference to 100 g. formula No. 1 assumes the following form:

$$\log E_{100} = \frac{100^{2/3} \log E_2}{a^{2/3}} = \frac{21.54 \log E_2}{a^{2/3}}.$$

It is obvious from the notation that the results of the measurements should be substituted into the formula in the form of the dielectric constant (E_2) of the powder poured into the condenser. The reading obtained in scale divisions cannot be applied directly. Therefore, prior to the measurements, it is necessary to calibrate the variable condensers in terms of the dielectric constant under the same conditions as will be applied in the subsequent work. The simplest method of calibration is to use fluids of known dielectric constant, which are consecutively poured into the measuring condenser, plotting the condenser readings obtained on a graph.

The liquids used for the calibration can be prepared very simply by mixing two organic liquids, one of which has a lesser and the other a greater dielectric constant than that which is required for the calibration. This measure is, however, advisable only if it is possible to make an accurate measurement of the dielectric constants of the mixtures thus prepared. If this is not possible, then we may use pure organic liquids the dielectric constants of which are known for definite conditions of measurement.

We generally used the following pure liquids:

Petroleum ether	dielectric constant 1.88	at 20°C.
Cyclohexane	dielectric constant 2.005	at 20°C.
Benzene	dielectric constant 2.28	at 20°C.
Toluol	dielectric constant 2.38	at 20°C.

and also mixtures of toluol and petroleum ether, and toluol and 96% ethyl alcohol.   On mixing small quantities of ethyl alcohol with toluol it was found necessary to add a little dioxane (diethylene dioxane, dielectric constant 2.23) in order to remove the turbidity due to the separation of water.

It should be mentioned that for practical purposes very great accuracy is not necessary in making the measurements of the absolute value of the dielectric constant of biscuit.   If the error is systematic, *i.e.*, if the standard and measuring condensers are of sufficient mechanical strength and the scale reading always corresponds to the calibration, then a difference between the measured value of the dielectric constant and the true value of some 0.02 to 0.03 will not be of vital importance. Particular attention need be directed only to the identity of the calibration liquids on making a repeated calibration, a verification, or a transition from one condenser or instrument to another.   The problem of these cases will be considered below.   Particularly careful purification of the calibrating fluids using very laborious methods is therefore not justified in practice.

With each of our 25 specimens of biscuit the dielectric constant was measured and recalculated to 100 g. five times.   The data obtained for 100 g. were plotted, the moisture contents being laid out as abscissas and the calculated values of the dielectric constants as ordinates.   The curve is shown in Figure 1.   This curve is of the same character as
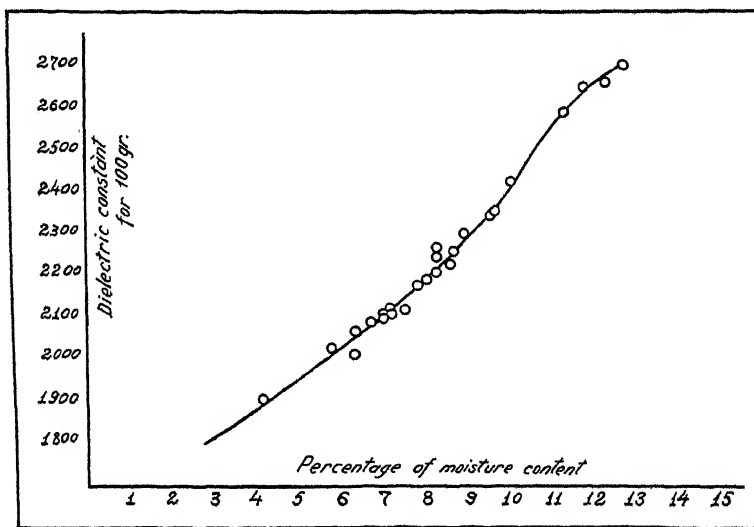

Fig. 1.   Curve of the dielectric constant of sweet biscuit as a function of moisture content for a weight of 100 grams.

that obtained in the preliminary experiments; it rises at first slowly and then more rapidly as the moisture content is increased. The upper bend cannot be considered as altogether reliably established owing to its position and to the small number of experimental points through which it is drawn. This curve may be used for practical determinations of the moisture content of biscuit if the latter is ground, poured into the condenser, weighed, and if the dielectric constant obtained is recalculated to refer to 100 g. However the necessity for recalculation, which incidentally is quite complicated, is of great inconvenience for practical work and requires considerable time and a certain degree of experience. In order to avoid this objection and to render the method truly rapid we decided to draw on the same co-ordinates a curve of the dependence of the dielectric constant on the weight of biscuit. The problem therefore consists in constructing a graph to express a function of three variables, the moisture content, the dielectric constant, and the weight of biscuit. For this purpose we chose the method of constructing a family of curves of dielectric constant vs. moisture content with the biscuit weight serving as a parameter characterizing the individual curves. If the weight is taken as the parameter which varies by steps, the necessary family of curves may be constructed by plotting, along with the curve relating to 100 g., curves for other weights.

It is inadvisable to calculate the data for these curves from the initial measurements on the specimens of biscuit substituting another weight into the formula in place of 100 g. It is much more convenient, proceeding from the curve already plotted (Fig. 1), to mark on it a number of points, read off the corresponding moisture content and dielectric constant, and then recalculate the data for the corresponding weights. We carried out this recalculation for 105, 110, 115 g. and so on up to 140 g. with 5-g. intervals. In order to recalculate for 105 g. the following numerical values are substituted into formula No. 1:

$$\log E_{105} = \frac{105^{2/3} \log E_{100}}{100^{2/3}} = 1.0334 \log E_{100}.$$

The factor 1.0334 remains constant for all values of the dielectric constant (E_{100}). Consequently the recalculation is quite simple. The factors obtained in this way for recalculation to 110, 115 g. etc., are given in Table I.

The moisture content and dielectric constants corresponding to the points taken on the curve for 100 g. and the data after recalculation to the various weights are given in Table II. In order to simplify somewhat the construction of the curves the points were chosen with moisture-content intervals of 1%.

TABLE I

CALCULATIONS FOR SAMPLES OF VARIOUS WEIGHT

Weight to be recalculated	Factor
g.	
105	1.0334
110	1.0659
115	1.0980
120	1.1295
125	1.1606
130	1.1913
135	1.2219
140	1.2521

TABLE II

DIELECTRIC CONSTANTS FOR VARIOUS WEIGHTS

Moisture content %	100 g.	105 g.	110 g.	115 g.	120 g.	125 g.	130 g.	135 g.	140 g.
3	1,810	1,846	1,882	1,919	1,955	1,989	2,028	2,065	2,102
4	1,870	1,910	1,949	1,988	2,028	2,068	2,108	2,149	2,190
5	1,943	1,987	2,030	2,074	2,118	2,162	2,206	2,252	2,297
6	2,020	2,068	2,116	2,164	2,213	2,261	2,311	2,361	2,412
7	2,105	2,158	2,211	2,264	2,318	2,383	2,427	2,483	2,540
8	2,200	2,259	2,317	2,377	2,437	2,497	2,558	2,621	2,684
9	2,310	2,376	2,441	2,508	2,574	2,643	2,711	2,782	2,853
10	2,440	2,514	2,588	2,663	2,739	2,816	2,894	2,974	3,055
11	2,590	2,674	2,758	2,843	2,930	3,018	3,107	3,191	3,292

These data were used in the construction of the family of curves illustrated in Figure 2. There is an individual curve for each of the weights for which the recalculation was carried out. The curves for intermediate weights may easily be obtained by simple graphical interpolation.

To effect a final simplification of the work of transition from dielectric measurements to moisture content by means of these curves, additional notation was introduced into the latter. First, in order to avoid the necessity of recalculating the reading of the " Dielkometer " to the absolute value of the dielectric constant we have laid out on the ordinate, together with the values of the dielectric coefficients, the corresponding scale indications of the instrument. The figures are taken from the calibration curve for the given measuring condenser. Finally, at the upper ends of the curves we have given the weight of the biscuit plus that of the condenser. This avoids the necessity of subtracting the weight of the empty condenser each time. As a result of these various measures the process of determining the moisture content from the dielectric constant in the case of ground biscuit requires very little time.
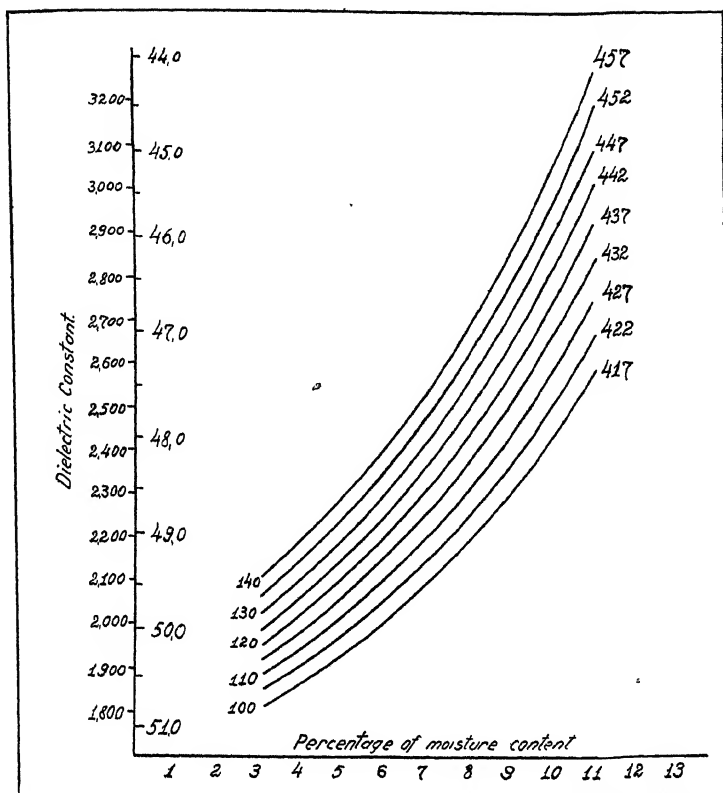
Fig. 2. Curve of the dielectric constant of biscuit as a function of moisture content for
different weights.

To carry out a determination it is only necessary to pour the ground
biscuit into the condenser up to the mark, pack it down somewhat by
tapping the condenser, insert the condenser into the instrument to take
the reading, and weigh the condenser on a balance with an accuracy of
0.3–0.5 g. From the point on the ordinate which corresponds to the
" Dielkometer " reading obtained it is then necessary to draw a line
parallel to the abscissa and on the curve corresponding to the appropriate
weight of biscuit to read off the corresponding moisture content on the
abscissa. This can all be done in 3 to 5 minutes.

Control Experiments

In order to determine to what extent the foregoing method is prac-
tically applicable, to find the degree of reproducibility when using the
family of curves for different weights, and to find the accuracy with

which the moisture content was determined, we carried out control measurements of moisture contents of 20 specimens of sugar biscuits simultaneously by means of a drying oven and the "Dielkometer." The method of carrying out the measurements was the same as in determining the curves. The data are given in Table III.

The arithmetical mean of the average divergences between the results of the measurements obtained from the dielectric constant and those obtained with the drying oven is 0.14% of moisture. The average deviation in each case does not, as a rule, exceed 0.15% to 0.20% of moisture. There were, actually, only two exceptions to this. The divergences between repeated determinations of the moisture content from the dielectric constant do not, in most cases, exceed 0.15% to 0.20%. Taking into account the rapidity of the analysis, these results may be

TABLE III

APPLICATION OF THE METHOD TO 20 BISCUIT SAMPLES

Exp. No.	Exp. temp.	Moisture content, desiccation method	Dielectric const. from instrument reading	Weight held by condenser	Dielectric const. referred to 100 g.	Moisture content, from dielectric const. curve		Divergence	Divergence mean of 3 results
	°C.	%		g.		%	Mean	%	%
1	22°	6.82	2.477	138.0	2.08	6.75	—	−0.07	—
2		6.82	2.435	133.3	2.09	6.85	6.78	+0.03	−0.04
3		6.82	2.444	134.9	2.08	6.75	—	−0.07	—
4	22°	8.07	2.531	129.5	2.18	7.85	—	−0.22	—
5		8.07	2.545	128.4	2.20	8.10	7.98	+0.03	−0.09
6		8.07	2.556	130.7	2.20	8.10	—	+0.03	—
7	22°	8.45	2.663	131.7	2.26	8.50	—	+0.05	—
8		8.45	2.658	132.4	2.25	8.50	8.48	+0.05	+0.03
9		8.45	2.636	131.2	2.24	8.45	—	−0.00	—
10	19°	12.34	2.991	117.3	2.68	11.70	—	−0.64	—
11		12.34	2.982	116.5	2.68	11.70	11.73	−0.64	−0.61
12		12.34	3.041	117.0	2.72	11.80	—	−0.54	—
13	19°	7.88	2.553	128.5	2.21	8.15	—	+0.27	—
14		7.88	2.510	126.1	2.20	8.00	8.05	+0.12	+0.17
15		7.88	2.523	127.6	2.20	8.00	—	+0.12	—
16	19°	11.44	2.969	114.5	2.70	11.70	—	+0.26	—
17		11.44	2.934	114.9	2.66	11.50	11.58	+0.06	+0.14
18		11.44	2.910	113.2	2.67	11.55	—	+0.11	—
19	17.5°	5.97	2.335	139.0	1.98	5.55	—	−0.42	—
20		5.97	2.335	136.6	1.99	5.60	5.58	−0.37	−0.39
21		5.97	2.324	136.0	1.99	5.60	—	−0.37	—
22	18°	9.13	2.630	125.4	2.30	8.90	—	−0.23	—
23		9.13	2.619	124.3	2.30	8.90	8.93	−0.23	−0.20
24		9.13	2.682	127.2	2.32	9.00	—	−0.13	—
25	19°	9.72	2.706	121.1	2.40	9.75	—	+0.03	—
26		9.72	2.754	123.5	2.41	9.80	9.78	+0.08	+0.06
27		9.72	2.774	124.4	2.41	9.80	—	+0.08	—
28	19°	8.10	2.553	128.8	2.21	7.95	—	−0.15	—
29		8.10	2.553	126.9	2.22	8.15	8.08	+0.05	−0.02

TABLE III—(*Continued*)

Exp. No.	Exp. temp.	Moisture content, desiccation method	Dielectric const. from instrument reading	Weight held by condenser	Dielectric const. referred to 100 g.	Moisture content, from dielectric const. curve		Divergence	Divergence mean of 3 results
	°C.	%		g.		%	Mean	%	%
30		8.10	2.545	126.3	2.22	8.15	—	+0.05	—
31	20°	6.01	2.324	129.9	2.03	6.10	—	+0.09	—
32		6.01	2.341	130.5	2.04	6.25	6.23	+0.24	+0.22
33		6.01	2.357	130.8	2.05	6.35	—	+0.34	—
34	20°	6.70	2.378	129.8	2.07	6.70	—	0.00	—
35		6.70	2.435	132.1	2.09	6.90	6.77	+0.20	+0.07
36		6.70	2.361	127.7	2.07	6.70	—	0.00	—
37	20°	5.50	2.269	130.9	1.98	5.55	—	+0.05	—
38		5.50	2.269	129.9	1.99	5.60	5.58	+0.10	+0.08
39		5.50	2.269	130.3	1.99	5.60	—	+0.10	—
40	20°	6.78	2.313	122.4	2.08	6.75	—	−0.03	—
41		6.78	2.324	123.3	2.08	6.75	6.77	−0.03	−0.01
42		6.78	2.322	122.3	2.09	6.80	—	+0.02	—
43	20°	6.44	2.313	124.0	2.07	6.65	—	+0.21	—
44		6.44	2.304	124.4	2.06	6.55	6.62	+0.11	+0.18
45		6.44	2.313	123.3	2.07	6.65	—	+0.21	—
46	20°	7.85	2.444	118.4	2.22	8.10	—	+0.25	—
47		7.85	2.424	117.2	2.22	8.10	8.05	+0.25	+0.20
48		7.85	2.378	115.0	2.20	7.95	—	+0.10	—
49		7.80	2.444	121.7	2.19	7.85	—	+0.05	—
50	20°	7.80	2.438	121.3	2.19	7.85	7.93	+0.05	+0.13
51		7.80	2.490	123.5	2.21	8.10	—	+0.30	—
52	20°	7.71	2.400	119.8	2.17	7.70	—	−0.01	—
53		7.71	2.413	121.1	2.17	7.70	7.70	−0.01	−0.01
54		7.71	2.398	119.8	2.17	7.70	—	−0.01	—
55	19°	7.86	2.400	117.6	2.19	7.95	7.90	+0.09	+0.04
56		7.86	2.422	120.6	2.18	7.85	—	−0.01	—
57	19°	8.49	2.575	124.5	2.26	8.60	—	+0.11	—
58		8.49	2.545	122.6	2.26	8.60	8.57	+0.11	+0.08
59		8.49	2.551	123.8	2.25	8.50	—	+0.01	—

considered as quite satisfactory and the " lightning " method of determining the moisture content from the dielectric constant is of quite sufficient accuracy for practical use in manufacturing control of the moisture of biscuits.

Further Development of Method

Although the results of the experiments described above permit of the conclusion that the determination of the moisture content of biscuit from the dielectric constant is already far enough developed to be applicable for practical purposes, it is nevertheless not as yet sufficiently standardized. According to the method herein presented an individual curve of the dependence of the dielectric properties of the biscuit as a function of the moisture content must be drawn up for each instrument

and each measuring condenser. The experimenter is limited to that condenser for which the curve is already determined. If it is necessary to replace the condenser by one of another shape or size, or if the instrument becomes damaged or if the plates of the old condenser are renewed, all the previous work must be repeated from the very start. This is a great inconvenience which may sometimes lead to complications in the work. Further, this circumstance might serve as an obstacle to extensive use of the method, since in production laboratories there is not always available a personnel sufficiently qualified to draw up an accurate calibration curve and to check on its reliability during subsequent use. It would be much more convenient if for a given type of product there were available a curve or system of curves established at some center and which were applicable for all cases of determination of the moisture content of this product from the dielectric constant. Such a curve might be obtained and used either through the availability of absolutely identical instruments and condensers, or by the development of a method of translation of results from one condenser to another. The manufacture of absolutely standard apparatus is so difficult that we considered it necessary to develop the method described below, which is based on principles already previously discussed.

A complicated question, for practical purposes, is the determination of the correction for differences between the temperature at which the calibration was carried out and that at which the measurement takes place. In conditions of manufacture it is not always possible to carry out the measurements at a temperature which is very close to the temperature of calibration. A large deviation in temperature may introduce a very considerable error into the moisture content determination.

Furthermore, it is very desirable to simplify the calculation of the measurement of the dielectric constant to one and the same packing density in the determination of the initial curve. Since we carried out all the subsequent work using a simpler method of calculation it is desirable to begin our further discussion of the method with this feature.

Simplification of the Calculations for Drawing Up the Calibration Curve .

Examination of formula No. 1 shows that the dielectric constant (E_1 and E_2) stands under the logarithmic sign on both sides of the equation. Hence it is clear that there will be rather less calculation to carry out if as the basic magnitude we take not the dielectric constant (E) but its logarithm to the base 10 ($\log E$). The substitution should be introduced at the very start, from the drawing up of the curve for transition from the readings of the " Dielkometer " to the absolute value of the

dielectric constant with a given measuring condenser as determined from the calibrating liquids.

Instead of plotting as ordinates the values of the dielectric constants of these liquids, the *logarithms* of their dielectric constants should be plotted. Figure 3 is an example of such a curve for the condenser with which our basic work was carried out. The corresponding value of log E may be found from this curve for any reading of the standard condenser. The value of the logarithm which is found is substituted
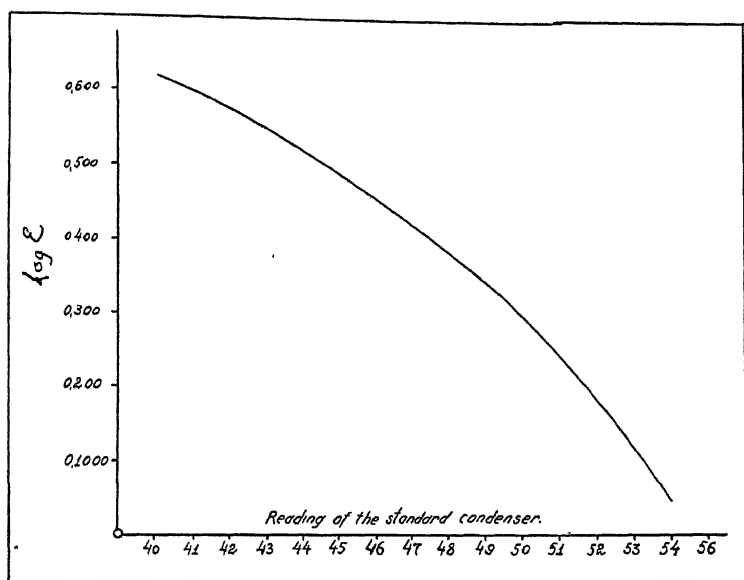
Fig. 3. Calibration curve for log E.

directly into formula No. 1. Using this method the number of times necessary to take logarithms is reduced by one.

If $L_1 = \log E_1$ and $L_2 = \log E_2$, then formula No. 1 assumes the following form:

$$L_1 = \frac{a_1^{2/3} L_2}{a_2^{2/3}}.$$

From the recalculation we obtain the logarithm of the dielectric constant corresponding to the weight to which all the data must be referred and thus avoid an expenditure of time in finding the number from its logarithm. Curves of the dependence of the logarithm of the dielectric constant on the moisture content of biscuit for different weights should be drawn up in the same way as before.

The recalculation becomes more simple if the entire work of calculation is replaced by the use of nomograms. We drew up a simple nomogram as shown in Figure 4 for referring the dielectric constant of biscuit for weights of 80 to 140 g. to a weight of 100 g. Certain changes were introduced into the basic formula for the purpose of drawing up this nomogram. The value of n in formula No. 1 was taken as 0.7 instead of 2/3. Experience shows that in this case the difference between the various weights of samples is perhaps even better than when we used $n = 2/3$.[4] The absolute values of the dielectric constant are somewhat altered but the calculation of nomograms becomes simpler.

Since almost all the experiments described below were carried out with the aid of nomograms it is necessary to bear in mind that the absolute values obtained in these experiments are incomparable with those obtained in the previous ones.

The formula was converted in the following manner for the purpose of drawing up the nomograms:

$$\frac{\log E_1}{\log E_2} = \frac{a_1^{0.7}}{a_2^{0.7}},$$

or

$$\log \log E_1 - \log \log E_2 = 0.7 \log a_1 - 0.7 \log a_2,$$

and for $a_1 = 100$ g., $\log \log E_1 - \log \log E_2 = 1.4 - 0.7 \log a_2$. The equation thus obtained affords the simplest means of obtaining relations between the three magnitudes E_1, E_2, and a_2 by means of three parallel scales. The method of constructing these nomograms may be found in any textbook on the construction of nomograms. In accordance with the usual method for using nomograms the measured value of the dielectric constant read off on the left-hand scale is connected by a straight line with the value corresponding to the weight on the right-hand scale, and the intersection of this line with the center scale gives the required value of E for 100 g.

Even though our nomogram was primarily intended for recalculation of the weights to 100 g., its applicability is not limited to this. From the relationship:

$$\frac{\log E_1}{\log E_2} = \frac{a_1^{0.7}}{a_2^{0.7}} = \left(\frac{a_1}{a_2}\right)^{0.7},$$

it is clear that the absolute values of a_1 and a_2 play no part and it is immaterial whether the value of the dielectric constant is recalculated from 80 g. to 100 g. or from 40 g. to 50 g., etc., provided the ratio of the values is constant. Therefore, on the right-hand scale of the nomogram we may replace the value of 100 g. by any other number, for

[4] We verified the applicability of formula No. 1 with $n = 0.7$ also for determining the moisture content of black tea, which enlarges the field for its use.

instance 20, while the remaining scales increase or decrease in the same proportion as the new number compares with 100. In this example it would be 100/20 equals 5. The nomogram may then be used to recalculate the data to the new weight (20 g.) instead of to 100 g.

Further, the nomogram may be used for the reverse recalculation from 100 g. to any other weight from 140 to 80 g., which is necessary for instance in drawing up the systems of curves in Figure 2. For this purpose the weight for which the recalculation is to be made is taken on the right-hand scale, the dielectric constant for 100 g. on the center scale, and the desired value of the dielectric coefficient is read off the left-hand scale.

Transition from One Condenser to Another

The final result of the measurement of the dielectric properties of powdery substances by our method is determined by two factors, the dielectric constant as obtained from the reading of the instrument, and the correction for variations in the density of packing. Therefore, on transition from one condenser to another it is necessary, in order to obtain comparable results from the measurements, that comparable results be obtained on measuring each of these factors individually. Identity of purely electrical measurements of the dielectric constant may be obtained by calibrating the condensers under perfectly identical conditions with calibrating liquids at a given temperature.

The following points must be taken into account in considering the density of packing: In the method which we have used the density of packing is governed by the weight of the substance required to fill the condenser to a given volume. This method, which is the simplest and most convenient for practical purposes, is applicable only to each condenser individually, since the weight of the substance required to fill the condenser depends on the size of the vessel in which the plates are fixed. If the density of packing is constant the quantity of substance which the condenser will hold is directly proportional to its volume. Therefore, on transition from one condenser to another it will primarily be necessary to take into account the ratio of the volume for holding powder in the one condenser to that in the other and to alter proportionately the constant weight to which all the results are referred. Suppose for instance that it is necessary to compare the density of packing in two condensers, one of which is of a volume of 237 cc. and the other 185 cc. If in the case of the first condenser all the results were referred to 100 g., then to obtain comparable results with the second condenser the data should be referred to a weight of

$$\frac{100 \cdot 185}{237} = 78.06 \text{ g.}$$

While this method is quite suitable for practical purposes, nevertheless it is more convenient to carry out the recalculation always to one and the same weight. For this purpose it is necessary to introduce a correction, not in the standard weight, but in the weight of powder which is poured into the condenser, which must be adjusted in accordance with the change in volume. If in the above example it is desired to effect the reference to 100 g., one should proceed in the following manner: Suppose that 69.4 g. of the investigated powder are required to fill the second condenser. We will calculate the weight of powder which would have been required to fill the first condenser to the same packing density. The volume of the first condenser is $237/185 = 1.281$ times greater than the volume of the second condenser. Consequently the weight required to fill the condenser with the same density of packing must be multiplied by the same factor, $i.e.$, $69.4 \times 1.281 = 88.9$ g. This is the weight which must be used in making the calculations.

This method has the advantage that the nomograms already prepared can be used for the final calculations of the dielectric constant. However the most general and simple method is to refer the results not to some definite weight required to fill the condenser up to a certain mark, but to the weight of powdery dielectric which occupies unit volume. The value obtained in this case characterizes the packing density uniquely, independently of the type of condenser. There can be no difficulty in calculating this value. Knowing the weight of powder and the volume of the measuring condenser and dividing the one by the other we easily obtain the desired result.

If a_1 and a_2 are weights required to fill the condenser to two different packing densities and v is the volume of the condenser, then formula No. 1 may be written:

$$\log E_1 = \frac{\left(\dfrac{a_1}{v}\right)^n \log E_2}{\left(\dfrac{a_2}{v}\right)^n} = \frac{p_1^n \log E_2}{p_2^n}, \tag{2}$$

where p_2 represents the packing density obtained at the time of measurement and p_1 the packing density taken as standard.

The nomogram illustrated in Figure 4 may be altered to correspond with this. As the starting point we may take formula No. 2, which is transformed to:

$$\log \log E_1 - \log \log E_2 = 0.7 \log p_1 - 0.7 \log p_2.$$

The actual numerical value to which the results are necessarily referred in this case, as before, depends on the way the investigated substance packs into the condenser. In our condenser, with 100-g. packing den-

sity, the quantity of biscuit in unit volume (1 cc.) was 0.422 g. of powder. Hence, in order to deal with round numbers, the reference may be made to a packing density of, for instance, 0.45, which leads to the following formula for the nomogram:

$$\log \log E_1 - \log \log E_2 = 0.7 \log 0.45 - 0.7 \log P_2.$$

In order to be able to judge to what extent the described method of transition from one condenser to another is practical we measured the dielectric constant of biscuit simultaneously with two condensers of very different sizes. The first condenser was the same one as was used in the basic measurements; its volume was 237 cc. The second condenser was of similar design to the first, but its volume was only 62.7 cc.

The second method of reducing the results to comparable data was used. The weight of powder required to fill the second condenser was multiplied by $237/62.6 = 3.78$ and the calculation was then effected by means of the nomogram illustrated in Figure 4. Since these methods
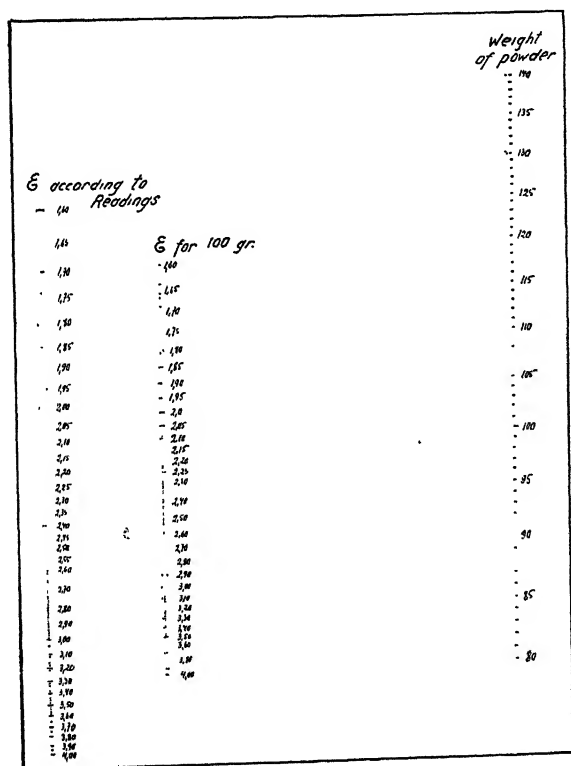

Fig. 4. Nomogram for recalculation of the dielectric constant of powder to a weight of 100 grams.

differ from each other only in the practical method of calculation, the results of the experiments should be representative of all of them. The calibration of the condenser was carried out with the same calibrating liquids as before at a temperature of 25°C. The biscuit was ground with a mortar and pestle, carefully mixed, and poured into the two condensers in turn. The measurements were carried out at different temperatures and with different specimen moisture contents. The numerical data are given in Table IV. It should be borne in mind that each figure is an average of five values obtained or calculated from experimental data.

TABLE IV

DETERMINATIONS WITH CONDENSERS OF DIFFERENT CAPACITY

No.	Temp.	Moisture content, drying cupboard	Condenser 1			Condenser 2				Diff. (100 g.) 1st and 2nd condensers
			Mean weight to fill	Mean dielectric const., from instrument	Mean dielectric const., after reference to 100 g.	Mean weight to fill	Mean weight after multiplication by 3.78	Mean dielectric const., read off instrument	Mean dielectric const., after reference to 100 g.	
	°C.	%	g.			g.	g.			
1	25	8.31	117.7	2.62	2.37	31.5	118.9	2.62	2.35	−0.02
2	25	6.05	111.3	2.25	2.12	29.2	110.3	2.24	2.13	+0.01
3	25	8.61	117.7	2.65	2.39	31.6	119.6	2.65	2.37	−0.02
4	25	7.35	116.8	2.43	2.21	31.1	117.2	2.43	2.21	−0.00
5	17	8.60	114.9	2.56	2.36	30.3	114.5	2.59	2.38	+0.02
6	24	9.37	.114.9	2.72	2.49	30.2	114.3	2.74	2.51	+0.02
7	14	8.21	121.1	2.54	2.26	31.1	117.5	2.51	2.27	+0.01
8	15	7.16	119.8	2.37	2.15	31.5	119.2	2.37	2.15	0.00
9	15	6.17	112.2	2.17	2.05	26.4	100.1	2.06	2.06	+0.01
10	13	8.02	115.8	2.43	2.23	30.9	116.7	2.48	2.26	+0.03
11	14	8.02	119.7	2.49	2.24	32.0	121.1	2.55	2.26	+0.02
12	15	8.40	122.8	.2.60	2.29	33.1	125.1	2.70	2.30	+0.01
13	16	7.61	121.8	2.48	2.22	32.5	123.7	2.56	2.24	+0.02
14	18	7.80	128.7	2.66	2.28	34.4	128.1	2.71	2.30	+0.02
15	20	10.80	114.9	2.98	2.70	31.1	119.1	3.05	2.71	+0.01
16	25	8.38	121.3	2.68	2.37	32.0	120.9	2.69	2.38	+0.01
17	.25	8.12	117.7	2.55	2.31	31.8	120.3	2.61	2.33	+0.02
18	25	7.06	120.9	2.48	2.22	33.1	125.1	2.54	2.23	+0.01
19	20	8.61	123.1	2.69	2.36	33.8	127.7	2.75	2.35	−0.01

The difference between the dielectric constants referred to the same packing density does not, as a rule, exceed 0.01 to 0.02 units of dielectric constant and the method described for transition from one condenser to another may be considered as suitable for practical purposes. The same methods may also be applied when it is necessary to change over from one instrument to another.

The methods described make it possible to use curves of the moisture content of the substance (as a function of its dielectric properties) established at different laboratories with different instruments and condensers. For this purpose it is only necessary to calibrate the instrument with the measuring condenser using the calibrating liquids, which is far easier than it is to determine the entire curve. We again emphasize that particular attention should be directed to identity of the standard calibrating liquids and of the conditions of calibration in the two cases.

Influence of Temperature on the Results of the Measurements

Investigators who have studied methods of using dielectric measurements to determine the moisture content of substances have noted the great influence of temperature on the results. In view of the inconvenience and often the impossibility of maintaining the required temperature under manufacturing conditions it is absolutely necessary to determine the correction for deviations in the temperature from the standard value. The influence of the temperature on the moisture determination is a total of the influences of the temperature on the measuring instrument and on the condenser and of the influence of the latter on the dielectric properties of the substance. Since our practical objective was to determine the value of the correction for our conditions, we were not interested in each of these individual factors, although it would be worth while to investigate each of them separately. Therefore, in working with biscuit, the temperatures of the instrument and the substance measured were altered simultaneously.

During the course of the experiments the "Dielkometer" was situated in a wooden thermostat which was insulated on the outside with asbestos. Heating was effected with an incandescent lamp, and the required temperature was maintained (by means of a thermoregulator) to an accuracy of $\pm 0.2°C$. Uniformity of the temperature throughout the thermostat was attained by use of a table fan. Calibration of the condenser with the standards liquids was carried out at 25°C. Measurements on specimens of ground sugar biscuit of different moisture contents were carried out at temperatures of 15°, 20°, 25°, and 35°C.

In view of the possibility that their moisture content might change if the specimens were to be poured several times, the determination of the moisture in the drying oven was carried out before each series of measurements. The instrument, the measuring condenser, and the specimens of biscuit contained in vessels with ground-glass stoppers were maintained in the thermostat at the given temperature for not less than 10 to 12 hours.

The results of the measurements were plotted with the dielectric constants of the powders related to 100 g., using the formula with $n = 0.7$ laid out on the vertical axis and the moisture contents on the horizontal one.   A curve for the relation of the dielectric constant of the biscuit to the moisture content was drawn in the usual manner through the experimental points for each of the temperatures used.   The points obtained, together with the corresponding curves, are shown in Figure 5.
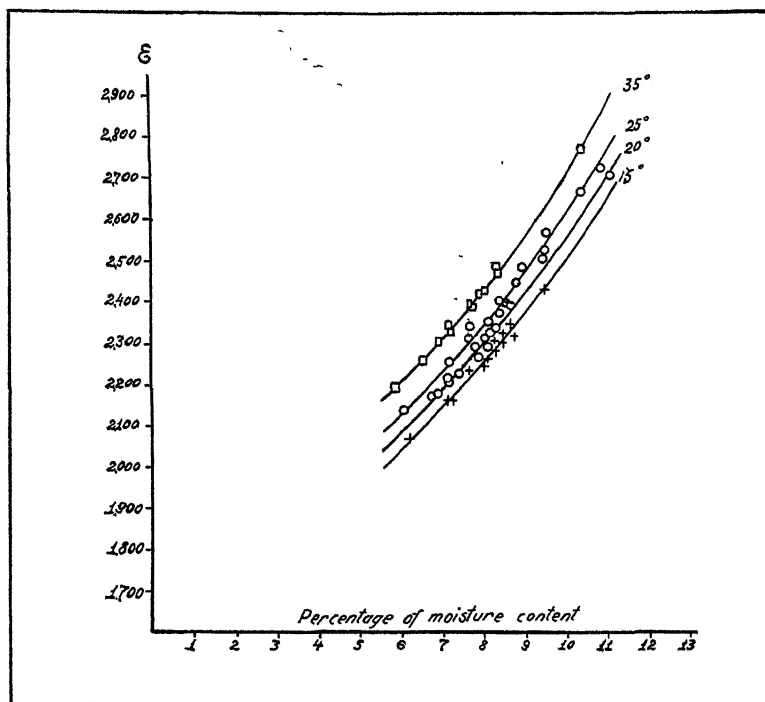
Fig. 5.   Curve of the dielectric constant of biscuit as a function of moisture content under different temperatures.

There is observed a clear and regular dependence of the dielectric properties of the biscuit on the temperature.   The higher the temperature of measurement the higher the position of the curves.   In using these curves it is necessary to bear in mind that they express the total dependence of the instrument readings and of the substance properties on the temperature.   Therefore, in principle, these curves are only suitable for practical application to the instrument for which they were drawn up and may be used only with a certain degree of approximation with other instruments of the same manufacture.   For instruments

which are very different in design from the " Dielkometer " these curves may be altogether unsuitable.

Comparing the positions of the curves illustrated in Figure 5 it will be seen that when the temperature changes by 10°C. the dielectric constant of the powder changes by 0.08 to 0.11 unit, the temperature having a greater influence the greater the moisture content. This corresponds to an error in the determination of the moisure content of approximately 0.7 to 0.08% ; *i.e.*, the correction per 1°C. is equal to about 0.07% to 0.08% of moisture content.

Concerning the Recalculation Formula

The main difference between the trend of our work and that of others who have studied problems of the determination of the moisture content of powders consists in the mathematical levelling of the packing density by means of an equation deduced on the basis of Lichtenecker's logarithmic law of mixtures:

$$\log E_1 = \frac{a_1{}^n \log E_2}{a_2{}^n}.$$

There are four magnitudes in the right-hand side of this equation of which three are obtained experimentally and one, n, is a constant.

It was found experimentally that the best agreement between results for different weights recalculated to 100 g. was obtained with n equal to between 0.65 and 0.7. There is little reason to suppose that the absolute value of n may be the same for other substances. Essentially, by changing the value of n which is the power to which the weight is raised, we attribute greater or less significance to the density of packing as one of the conditions which determine the dielectric constant of the powder. The graph given in Figure 6 shows how greatly this value changes. This graph shows the change in the value of the dielectric constant obtained from formula No. 1 with various values of n from 0 to 1 with weights of from 80 to 140 g. referred to 100 g., when the measured value of the dielectric constant is 2.0. The values of n are plotted on the abscissa and those of the dielectric constant on the ordinate. As n is increased the curves diverge increasingly. For instance if $n = 0.1$ and the weight of the biscuit is 120 g. recalculation gives a value of the dielectric constant of 1.975; with the same weight of biscuit and $n = 0.5$ the dielectric constant is 1.883; while for $n = 1$ the dielectric constant is 1.782. When $n = 0$ the weight plays no part at all.

Since the weight determines only the quantity of substance, not its quality and structure, it is in the present case a purely conditional magnitude which is easily measured in practice and its relative importance in

determining the value of the dielectric constant will obviously vary with alteration in the composition and properties of the powdery dielectric. A consequence of this is that it may be necessary to change the value of n for different materials in order to obtain the best results of recalculation. This fact is of immense importance for practical purposes. It may be supposed that one of the main factors which influence n will be the electrical conductivity of the powder which conditions the dielectric losses. We decided to verify this supposition in the following manner.
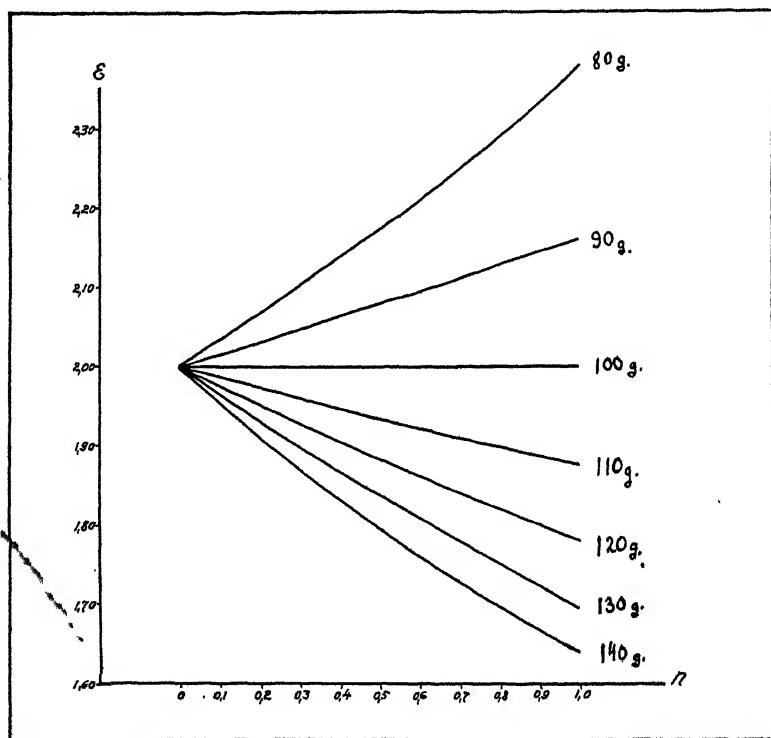
Fig. 6. Influence of the magnitude n on the results of the recalculation.

A specimen of tempered quartz sand, which had been very carefully washed free of all salts, was divided into several parts. Into each of these specimens we introduced standard quantities of KCl solution of different concentrations. It is clear that the greater the concentration of the KCl the greater the electrical conductivity of the mixture. The specimens of sand with different degrees of electrical conductivity thus prepared were poured into the measuring condenser by two methods: with very slight and with the densest possible packing which could be

obtained by shaking and tapping on the walls of the condenser. Readings were taken on the instrument in the usual manner, and afterwards the condenser was weighed. From the experimental values we calculated the value of n most suitable for recalculating the results.

The following equation for finding n was obtained by taking the logarithm of formula No. 1:

$$n = \frac{\log \log E_1 - \log \log E_2}{\log a_1 - \log a_2}.$$

Into this equation we substituted values for E_1 and E_2, a_1 and a_2, which were obtained from two measurements with one and the same specimen but with different densities of packing which were combined in such a way as to obtain the greatest possible difference between the densities of packing.

About 20 experimental measurements were taken for each test and several dozens of pairs of combinations were taken for calculation of the value of n. The arithmetic mean of the results obtained was then taken, and this was accepted as the most suitable value for n. The data are given in Table V.

TABLE V

VALUES OF n FOR SAND

Investigated substance	Average value n	Investigated substance	Average value n
Dry quartz sand	0.9906	Quartz sand with $0.02N$ KCl (3 cc. per 100 g.)	0.7089
Quartz sand with water (2 cc. per 100 g.)	0.9500	Quartz sand with $0.03N$ KCl (2 cc. per 100 g.)	0.6292
Quartz sand with $0.001N$ KCl (2 cc. per 100 g.)	0.9829		
Quartzs and with $0.01N$ KCl (2 cc. per 100 g.)	0.8117		

The dependence of the value of n on the electrical conductivity is quite clearly expressed. The greater the latter the less the calculated value of n. A certain inconsistency at the start may be due to experimental errors. The relationship shows that as the electrical conductivity is increased the weight of the quartz sand plays an ever smaller part; i.e., a lesser part is played by the quantity of dielectric, and other factors become more important. B. P. Alexandrov and G. P. Mikhailov [5] consider that the capacity of a condenser filled with a powdery dielectric is determined by a multiplicity of different capacities of the microcon-

[5] J. Tech. Phys. (Russia) 8 (No. 12): 1121 (1938).

densers which are formed within the space. Connection between these elementary condensers is established by bridges or films consisting of the aqueous solutions which are situated on the surfaces of and in the capillaries formed by the particles. Owing to their conductivity these solutions form, as it were, condenser plates separated by the particles of the substance from which the dielectric is formed. This conception may be particularly applicable to such powdery substances as quartz sand in which the water is situated on the surfaces of the particles. By varying the concentration of the solution added we alter the conditions of formation of the microcondensers, increasing them as the electrically conducting part is increased in comparison with the nonconducting. It is quite possible that this effect is the cause of the reduction in the part played by the weight, which in turn reduces the absolute value of n, since the volume of the actual dielectric, as it were, decreases during this process.

The structure and properties of such powdery substances as ground biscuit are very different from those of quartz sand. First of all they are able to retain water not only on the surface but are also able to absorb it within themselves owing to the large number of capillaries and to the colloidal properties of the substance. It is therefore to be expected that the relationships which are applicable because of a change in the electrical conductivity of biscuit will in this case be somewhat different from those in the case of quartz sand, and will undoubtedly be of greater or less influence.

We carried out the following experiments in order to make sure that electrical conductivity plays a part in measurements of the dielectric constant. First of all we prepared specimens of biscuit according to a recipe for one of the known types of sugar biscuits.[6] One specimen was prepared according to the usual recipe, the second was prepared without any addition of salt, and the third with five times as much of it as in the normal recipe. Each specimen was ground and divided into several portions of which each was then moistened to a different degree by placing over water in a desiccator.

There were thus obtained three series of samples with different moisture contents within each series. The dielectric properties of the specimens were measured in the usual manner, all the data being referred to 100 g. by means of the formula with $n = 0.7$. The results of the experiments expressed in the form of curves are given in Figure 7. The moisture contents of the specimens are laid out on the horizontal axis and on the vertical one the dielectric constant referred to 100 g. Not all the curves lie at the same level. Curve III for biscuit with a large

[6] Flour, 320 g.; sugar powder, 140 g.; invert sugar, 15 g.; maize starch, 24 g.; margarine, 60 g.; salt, 2 g.; soda, 2.2 g.; ammonia, 0.2 g.; essence, 1 g.; milk, 36 g.; melange, 24 g.

quantity of salt lies considerably lower than the others. The influence
of the great salinity is quite clearly expressed.

Curves I and II lie almost on the same level. The divergence be-
tween their upper parts is very likely incorrect and may be explained by
fortuitious experimental circumstances. In our opinion this coincidence
is due to the small difference between the compositions which differ by
only 2 g. of salt per 100 g. of sample. The sample without salt should
be more correctly designated as a specimen *without the addition of re-
fined salt*, since other salts are present in fair quantity. The addition
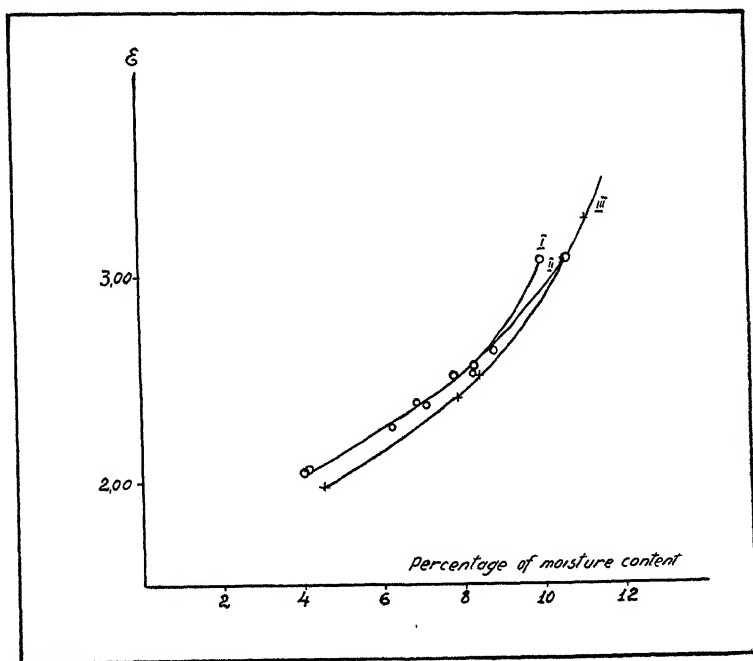
Fig. 7. Curves for biscuit with varied salt content.

of a five-fold quantity of salt causes an acute change in the ash content
of the biscuit, which has its influence on the results of the measurements.

The following two conclusions may be drawn from these experi-
ments: (1) The value of the constant n for which the best results for
leveling out the differences in the packing density are obtained may be
different for different substances. Preliminary experiments are neces-
sary in each individual case. (2) The small difference in the ash con-
tent (salinity) of the biscuit does not play an appreciable part.

Insofar as concerns the influence on the value of n of such other

factors as the structure and size of the particles of powder, the size and shape of the condenser and so on, it may be supposed that they also have a certain influence. This question requires further research.

Work with Granulated Sugar

Preliminary experiments carried out in the previous work demonstrated that the dielectric constant of sugar depends upon its moisture content, but it was not finally apparent which of the formulas was most suitable for taking into account the packing density of the granulated sugar in the condenser. The results obtained with Lichtenecker's formula and our own were almost identical.

Lichtenecker's formula is

$$\log E \text{ substance} = \frac{\log E \text{ mixture}}{\delta},$$

where E mixture is the dielectric constant of a mixture of the substance with air and δ is that fraction of the volume which is occupied by the substance. This formula corresponds to our formula:

$$\log E_1 = \frac{a_1^n \log E_2}{a_2^n}, \qquad \text{when } n = 1.$$

Recalculation when the index is equal to 1 is much simpler than for n greater or less than one, and it would therefore be convenient to apply such a formula.

In order to show the applicability of one expression or another for our purpose we carried out the following experiments. First we prepared specimens of granulated sugar with crystals of different sizes. For this purpose a certain quantity of commercial granulated sugar was divided into a number of fractions according to the size of the crystals, by passing it through metal sieves of different gauges. We thus obtained three samples of fine, medium, and coarse crystals. Finally, in order to obtain still finer crystals the sugar was ground to a powder with a mortar and pestle. The measuring condenser was filled up to the mark with these substances. It was then enclosed into the instrument and finally weighed.

The measured values of the dielectric constant were referred by the formula to the standard weight of 180 g. and according to the way in which the results agreed for different specimens we judged the advantage of one or the other formula. The data are given in Table VI.

Reference to the data for 180 g. shows that the use of the formula with $n = 1$ gave very good results. Repeated determinations carried out on one and the same specimen but with different packing densities coincide better than when $n = 2/3$.

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TABLE VI

MEASUREMENTS ON SUGAR PARTICLES OF VARYING SIZE

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Substance	Moisture content	Diel. const. from instrument	Weight of substance	Diel. const. of sugar at 180 g. calculated with $n = 2/3$	Mean	Diel. const. gran. sugar at 180 g. calculated with $n = 1$	Mean
	%	g.	g.				
Granulated sugar	0.01	2.169	221.0	1.964	1.962	1.873	1.882
		2.131	216.5	1.952		1.876	
		2.142	216.6	1.960		1.884	
		2.178	219.3	1.978		1.894	
		2.131	215.9	1.955		1.881	
Coarse grained fraction	0.01	2.035	200.0	1.940	1.926	1.896	1.882
		2.021	200.6	1.925		1.880	
		2.033	200.9	1.933		1.888	
		2.024	203.3	1.916		1.867	
		1.997	197.3	1.917		1.880	
Fine grained fraction	0.04	2.146	214.0	1.975	1.982	1.901	1.903
		2.207	217.5	2.011		1.926	
		2.166	216.5	1.981		1.902	
		2.149	215.3	1.972		1.897	
		2.164	218.0	1.972		1.891	
Sugar powder	0.02	2.144	213.0	1.977	1.961	1.905	1.887
		2.162	219.0	1.967		1.885	
		2.133	216.0	1.956		1.880	
		2.129	215.8	1.954		1.878	
		2.093	209.4	1.950		1.887	

The advantages of the second formula are still more obvious on comparing the data for specimens of different grain sizes. The concordance of values for the dielectric constant is very good in spite of the great difference between the weights of the substance required to fill the measuring condenser. Hence we may draw the conclusion that it is most advantageous to use the formula with the value of $n = 1$, in order to take into account the packing density of granulated sugar.

It is obvious that neither the electrical properties nor the ability of individual particles to be packed into the condenser are the same for biscuit and sugar. The results of the experiments with granulated sugar consequently confirm the conclusions of the previous section of this work.

Work with Flour

The work was carried out with specimens of 35% and 85% wheat flour. The experiments showed that the design of measuring condenser used for biscuits is not suitable for accurate measurements of the di-

electric constant of flour. The flour was not distributed uniformly throughout the condenser and formed into layers of greater or lesser density, sometimes even leaving spaces, which considerably reduced the reproducibility of results. The experiments should be continued with a condenser in which the distance between the plates is so great as not to hinder uniform distribution of the flour throughout the entire volume of the condenser.

Summary

The work was mainly devoted to verification of the applicability of dielectric measurements for the determination of the moisture content of ground biscuit and led to satisfactory results and to further methodological development of this procedure.

The question of the conditions for determination of the curve of the dielectric constant of the substance as a function of the moisture content was studied in detail.

A system of simplified calculations for practical measurements and for drawing up the curve by means of graphs and nomograms was worked out.

A method of transition from one condenser to another without lengthy calibration was developed and verified experimentally.

The question of the influence of the temperature on the results of the measurements was investigated.

A preliminary investigation was made of the question of the influence of the composition of the dielectric on the constant n in equation No. 1, used for recalculation.

A comparison was made between the applicability of formula No. 1 when $n = 2/3$ and when $n = 1$ for granulated sugar.

DOUGH IMPROVEMENT STUDIES. IV. EFFECT OF SOME FACTORS INVOLVED IN RESPIRATION ON THE BAKING QUALITY OF WHEAT FLOUR

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Since Pasteur's work it has been known that a relationship exists between fermentation and oxidation. The common substrate in both cases is a triose according to Szent-Györgyi (1937, 1938, 1938a). When oxygen is lacking fermentation takes place, two hydrogen atoms of the triose being transferred to pyruvic acid, which becomes lactic acid (*e.g.* in muscle). On the other hand in the presence of oxygen we have oxidation, of which respiration—the most powerful function in cell life—is one form.

Here the triose gives off two hydrogen atoms to oxalacetic acid, which Szent-Györgyi considers the most important hydrogen carrier in respiration. This acid is converted into malic acid. Both malic and oxalacetic acid are activated by the enzyme malicodehydrase, whereas succinic acid is activated by succinodehydrase. Fumarase, a powerful enzyme contained in all tissues, makes fumaric acid take up one molecule of water and become malic acid. Paradoxically, it also makes malic acid give off water. If one of these acids is added, in the presence of fumarase, an equilibrium (fumaric : malic = 1 : 3) is reached. Succinic acid is oxidized by cytochrome, while malic acid is oxidized to oxalacetic acid. Szent-Györgyi's scheme of H transport is:

Substrate (triose) → oxalacetic acid → [malic acid ⇌ fumaric acid] → succinic acid → WKS—O_2
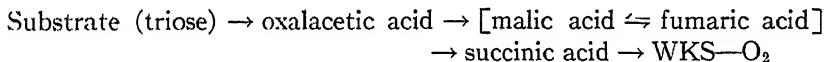
By "WKS" is meant the Warburg-Keilin system, *i.e.*, cytochrome and cytochrome oxidase.

Figure 1 shows the interrelationship of several acids involved in respiration and fermentation. Upward directions represent oxidations; those downward show reductions. Among substances which greatly increase respiration are fumaric and succinic acids (Szent-Györgyi, 1937) and adrenaline (Haurowitz, 1938). Substances known to decrease respiration are maleic acid (Thunberg), malonic acid (Quastel), and pyrophosphate (Dixon). The two last-mentioned compounds poison succinodehydrase. Cysteine is an inhibitor of yeast respiration.

Oppenheimer and Stern (1939) in their recent review of biological

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oxidation, are still unable to assign a place to glutathione in cell respiration.   The respiration of yeast or of animal tissues is affected neither by reduced (GSH) nor by oxidized glutathione (GSSG).   Kendall and Nord suspect an intermediary form between GSH and GSSG.   The hydrogenation by a donator and the dehydrogenation by an acceptor
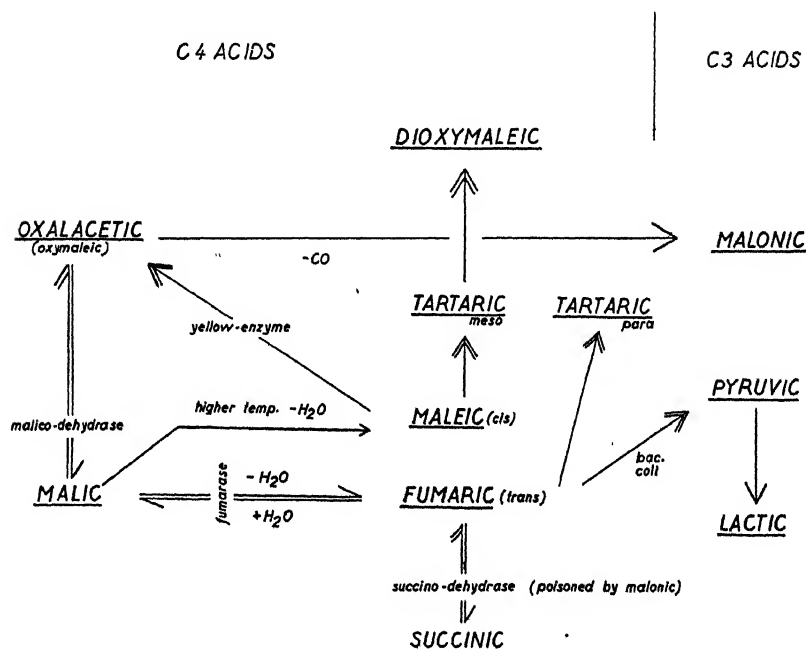
Fig. 1.   Interrelationship of some acids with three and four carbon atoms involved in respiration and fermentation.

seem to be more complicated here than in the case of other carriers. Oxidation processes of interest in studying flour quality might be summarized as follows:

Oxidation process	Main substrate in wheat flour affected	Effect in relation to baking as result of oxidation of substrate
Fermentation	Carbohydrates	Production of gas (CO_2)
Respiration	Carbohydrates	Unknown
Aging	(a) Proteins, glutathione	Improvement of gluten quality
	(b) Unsaturated fatty acids	Damage to gluten quality
	(c) Fats (development of rancidity)	Damage to keeping quality

Freilich and Frey (1939) have demonstrated that mixing in oxygen will partly eliminate the bad effect of germ, cysteine, and glutathione added to a dough.   The object of the present investigation was to

test whether the action of some factors involved in or influencing respiration can help to dehydrogenate (*e.g.*, glutathione) or to activate oxygen so that a higher degree of oxidation of the dough results, than that found by the authors mentioned. The tests might also help to explain the mode of action of oxidation in dough, known to lead to the improvement of bread.

Materials

Papain has recently been isolated in a pure state by Balls and Lineweaver (1939). Bergmann, Fruton, and Fraenkel (1937) believe that papain contains two enzymes, which they call papain I and papain II. It has been shown by Rupp and Bailey (1937) that small increments of papain did not cause any degradation in a dough.

Papain or papayotin is activated by glutathione due to the reduction of the enzyme itself (Bersin, 1933). Purr (1935) found that the enzyme is inactivated by alloxan. Papain is slowly destroyed in a pure state. It is not affected by aeration alone but aeration in the presence of copper and iron ions caused partial inactivation (Hellermann and Perkins, 1934).

Commercial preparations of papain contain sulphur in the form of cystine, methionine, and some unidentified compound (Schöberl and Fischer, 1939). At the same time several "impurities" are reported in commercial preparations: a natural papain activator, sterols, a lipase, a reducing dyestuff, carbohydrates, and zinc, the latter an enzyme poison (Schöberl and Fischer, 1939; Ganapathy and Sastri, 1938; Maschmann and Helmert, 1934; Sandberg and Brand, 1925).

For our work we used Merck's papain 1 : 20. The adrenaline used was a Merck product marked Ph. helv. V., which is the *l*-adrenaline base. The aneurin (thiamin) was also from the same firm. The malic acid was the inactive compound. The reduced glutathione and ascorbic acid were from Hoffmann-LaRoche in Basle (Switzerland).

The gluten flour came from a starch factory. It had retained its swelling power and contained 3.6% starch, 0.69% fat, and 8.2% moisture.

Methods

Titrations were carried out with $0.0020N$ iodine to determine the degree of oxidation of glutathione, as previously described (Ziegler, 1940, 1940a). It takes 1.63 cc. $n/500$ iodine to completely oxidize 1.00 mg. of reduced glutathione. The completely oxidized glutathione still needed 0.15 cc. of iodine. This method was shown to differentiate between the activity of bromate and the inactivity of chlorate as oxidizing agents of reduced glutathione.

Turbidity measurements were made by a method which is a com-

bination of the Berliner (1929, 1937) gluten swelling determination and of the method used by Flohil (1936), which enabled us to determine rapidly the activation or inhibition of papain action on gluten in the presence or absence of glutathione.   Flohil set away washed-out gluten (divided into small pieces) with a trace of boric acid for 48 hours at 30°C.   According to the substance added the supernatant liquid was more or less turbid after the two days.   To shorten the test we applied the Berliner method of allowing the gluten to swell and dissolve in $n/50$ lactic acid at 27° for 60 minutes in a rotating thermostat.   The turbidity was measured with a photoelectric cell after centrifuging. Objections have been raised against the Berliner method of determining gluten quality, mainly by Bungenberg de Jong (1939) on the grounds that it only considers one of the components.   As mentioned by Berliner (1931), gliadin goes into solution from all glutens during the test, but in the case of glutens classified as weak, a breakdown occurs at the same time, whereas strong glutens do not disintegrate.   Whatever the exact value of the Berliner method may be—we have always found it useful in classifying small samples of wheat—it proved very satisfactory for the present tests, in spite of the drawback of having to work in a strongly acid medium (pH 3).   Four different photometers, designated as B_1, B_2, L, and DC, were used so that all figures here reported cannot be directly compared.   Not every photometer with a photoelectric cell can be used for these measurements because of the

TABLE I

TITRATION OF MIXTURES OF GLUTATHIONE AND DIFFERENT AGENTS

(In each case 1 mg. reduced glutathione in 10 cc. distilled water.
Reaction time 1 hour.)

No.	Addition		$n/500$ iodine
		mg.	cc.
1	None	—	1.70
2	Potassium bromate	50	0.15
3	Ammonium metavanadate	5	1.40
4	Ammonium metavanadate	20	2.70 [1]
5	Fumaric acid	5	2.00
6	Fumaric acid	20	2.00
7	Succinic acid	5	1.75
8	Maleic acid	50	1.90
9	Malonic acid	5	2.30 [1]
10	Nicotinic acid amide	5	1.65

[1] Blue color disappears.

Tyndall effect; small concentrations of suspended particules throw a greater amount of light on the cell than would be the case when pure water is placed between the light source and the cell.   The photometer

can be built so that the smallest concentration of suspended particles corresponds to an increase in light absorption, as measured by the microammeter. That was the case with our measurements.

When gluten flour was used alone, only a slight turbidity was observed (*i.e.* low percentage of absorption). On adding a fair amount of protease a high absorption is found (Table II, 1–5). Figure 2

TABLE II

EFFECT OF DIFFERENT ADDITIONS ON THE TURBIDITY OF
GLUTEN FLOUR IN ACID SOLUTION

(0.5 g. gluten flour in 20 cc. *n*/50 lactic acid, shaken for 1 hour at 27°C.,
measurement in an electric photometer after centrifuging.)

No.	Papain		Addition	% light absorbed	Inhibition partial (*) or total (**)
	mg.	*mg.*		*Photometer B1*	
1	—	—		15	
2	5	—		17	
3	10	—		32	
4	20	—		73	
5	30	—		83	
6	—	7.5	Glutathione (reduced)	16	
7	30	—		83	
8	30	5	Potassium bromate	14	**
9	30	5	Potassium iodate	12	**
10	30	5	Potassium chlorate	83	
11	30	30	Ascorbic acid	82	
12	30	30	Dehydroascorbic acid	42	*
				Photometer B2	
13	20	—		92	
14	20	5	Potassium bromate	15	**
15	20	5	Succinic acid	93	
16	20	5	Fumaric acid	86	
17	20	5	Malic acid	88	
18	20	5	Maleic acid	43	*
19	20	5	Malonic acid	92	
20	20	5	Adrenaline	87	
21	5	—		24	
22	5	5	Glutathione (reduced)	87	
23	5	{5 / 5	Glutathione (reduced) Potassium bromate	14	**
24	5	{5 / 5	Glutathione (reduced) Maleic acid	30	*

shows the rise in light absorption with increasing amounts of papain for two photometers with somewhat different characteristics. As the white colloidal solution always remains translucent, there is always a certain amount of light which falls on the cell. This explains why the absorption measured never reached 100%, *i.e.*, complete darkness. Figure 3 shows the effect of activation of papain by increasing amounts of glutathione. Five mg. and 7 mg. of GSH gave 91% and 91.5%. Figure 4 was obtained by progressive dilution with *n*/50 lactic acid of
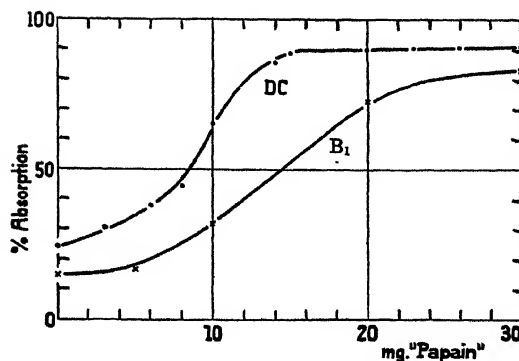
Fig. 2. Turbidity measurements with photometers DC and B₁. Different amounts of "papain" added to 0.5 g. of gluten flour. Distilled water taken as 0% absorption.
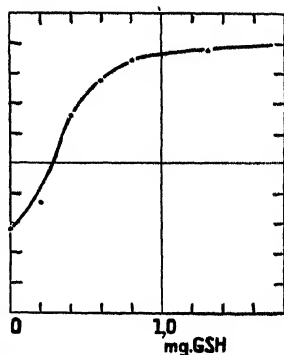
Fig. 3. Turbidity measurements with photometer DC. Different amounts of GSH added to 0.5 g. of gluten flour + 5 mg. papain.

one of the solutions used for Figure 3. The papain preparation did not contain any heat-stable activator, as even a large amount of papain after boiling for 30 minutes did not increase absorption (Table III, 34–37). In fact a slight decrease (Nos. 36 and 37) was measured which cannot yet be interpreted. Twenty mg. of boiled papain had no effect on 15 mg. of unboiled papain.

Figures 2, 3, and 4 show that, with photometer DC, in the presence
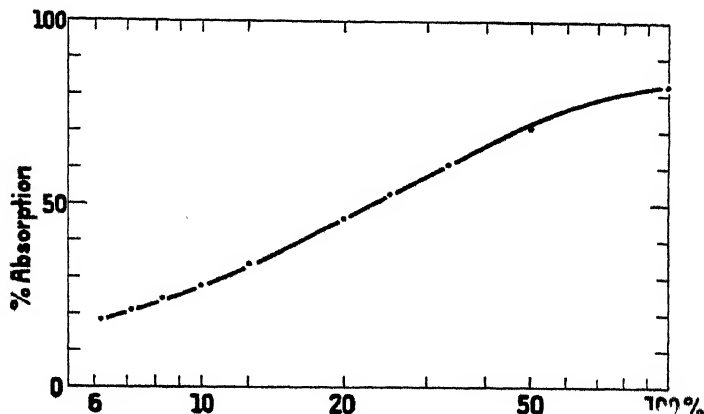
Fig. 4. The dilution of a solution from a turbidity test containing gluten flour, papain, and glutathione as a logarithmic function of absorption.

of not more than 15 mg. of papain, even the smallest increment of an inactivator could be measured. The same applies to the amount of glutathione in the presence of 5 mg. of papain. Here (Fig. 3) 1.5 mg. of GSH would be enough. We used 5 mg. of GSH throughout, be-

TABLE III

Effect of Different Additions on the Turbidity of Gluten Flour in Acid Solution

(0.5 g. gluten flour in 20 cc. $n/50$ lactic acid, shaken for 1 hour at 27°C. Turbidity measured with an electric photometer after centrifuging.)

| No.	Papain	GSH		Addition	% light ab-sorbed	Inhibition partial (*) or total (**)
	mg.	mg.	mg.	*Photometer L*		
1	–	–	—		31	
2	5	–	—		42	
3	5	5	—		94	
4	5	5	5	Potassium bromate	29	**
5	5	5	5	Potassium iodate	32	**
6	5	5	5	Potassium chlorate	93	
7	5	5	5	Potassium chlorate+osmic acid [1]	62	*
8	5	5	5	Ammonium persulphate	37	**
9	5	5	5	Ammonium metavanadate	28	**
10	20	–	5	Ammonium metavanadate	19	**
11	5	5	5	Alloxan [2]	72	*
12	5	5	5	Succinic acid	95	
13	5	5	20	Succinic acid	90	
14	5	5	5	Fumaric acid	95	
15	5	5	20	Fumaric acid	90	
16	5	5	5	Malic acid	96	
17	5	5	20	Malic acid	90	
18	5	5	5	Malonic acid	94	
19	5	5	20	Malonic acid	89	
				Photometer DC		
20	–	–	—		24	
21	5	–	—		36	
22	5	5	—		94	
23	5	5	5	Adrenaline (oxidized by heating)	79	*
24	5	5	5	Adrenaline (red)	94	
25	5	5	{5 / 5	Adrenaline (red) Copper sulphate	48	*
26	5	5	5	Copper sulphate	63	*
27	5	5	5	Aneurin (thiamin)	95	
28	5	5	5	Nicotinic acid amide	91	
29	5	5	5	l-asparagine	91	
30	5	–	5	l-asparagine	30	
31	5	5	5	"Chloramin" (Heyden)	90	
32	5	5	20	"Chloramin"	45	*
33	20	–	5	"Chloramin"	25	**
34	–	–	—		24	
35	–	–	20	Boiled papain	23	
36	5	–	—		30	
37	5	–	20	Boiled papain	26.5	
38	–	5	—		24	

[1] One drop 2% osmic acid.
[2] Not all dissolved.

cause we had carried out the preliminary tests in that way, but this implies that the oxidizing agents would first have to inactivate the surplus GSH before any oxidation of the glutathione could be detected by the photometer.

The tests and measurements were carried out in test tubes which, filled with water, differed to the extent of 0%–1% in absorption. The usual motor-driven centrifuge could not be used, so that we had to resort to a hand-driven centrifuge which could hold the test tubes. The irregular centrifuging accounts for most of the differences found when tests were duplicated.

Glutathione (GSH) alone (*i.e.*, in the absence of papain) had no effect on turbidity (Table II, 1, 6; Table III, 34, 38), from which we deduce that the gluten flour contained no active protease and was not attacked by GSH under these conditions. A similar test, but with 0.5 g. of fresh wet gluten alone and in the presence of 5 mg. of GSH, gave 22% and 26%. In the wet gluten no amount of active protease of any importance seemed to be present. A mixture of papain and glutathione was inactivated by the addition of bromate, iodate, persulphate, and metavanadate, whereas chlorate was without effect (Table III, 4–10). It has been shown (Ziegler, 1940a) that, by the titration method referred to above, chlorate in the presence of osmic acid will oxidize glutathione, an effect also revealed by the turbidity method (Table III, 6, 7). Metavanadate was included because its action has been studied in connection with protease (Read and Haas, 1938; Hale, 1939).

The baking tests were carried out with 200 g. of flour from a 1 : 1 mixture of Canadian and Argentine wheat, which could be improved by bromate. After mixing, the dough was divided into two parts. The dough contained 2% salt, 3% yeast, 0.5% maltose, but no fat. The mixing was done in a hand-driven iron mixer so as to avoid strong mechanical treatment and possible traces of copper, shown recently by Bungenberg de Jong and Klaar (1939) to be of importance when dough is mixed in oxygen. Even without oxygen a copper bowl can cause a reduction in loaf volume, as observed by Frank (1932). In all cases a current of oxygen was fed into the mixer, because the problem was to see whether respiratory carriers could be used to transport oxygen for the oxidation of dough.

Factors Involved in Animal Respiration

Three important factors in respiration are not included in this study, namely cytochrome, oxalacetic acid, and hemoglobin. The four substances mentioned below are all either easily reduced or oxidized. Bungenberg de Jong (1939) explained that in a dough it does

not seem to matter whether the reduced or oxidized form of such redox substances is added.[1]

Succinic acid (COOH.CH$_2$ CH$_2$.COOH).—No substance is oxidized by tissues as rapidly as succinic acid. If oxygen is present, succinic is oxidized to fumaric acid, but this reaction can be poisoned by malonic acid and by pyrophosphate (Szent-Györgyi, 1937). Succinic acid reduced cytochrome *c* much faster than any other substrate (Straub, 1937). The acid is activated by succinodehydrase (which will be referred to under malonic acid) and increases respiration. If succinate is added to tissue, more oxygen will be taken up than the tissue ever consumes in normal respiration (Szent-Györgyi, 1937). In plant respiration polyphenols take the place of this acid (Szent-Györgyi, 1938a).

Kent-Jones (1934) mentioned succinic acid as a useful and powerful improver. Alcoholic fermentation transforms about 0.01%–0.02% of a flour into succinic acid; the higher the percentage of yeast, the more succinic acid formed (Guillemet, 1937). The presence of ammonium chloride prevents this formation (Hoffman, 1917). Kent-Jones (1939) believes that the improving action of some substances may be due to the formation of abnormally large quantities of succinic acid during fermentation. There is however no direct evidence of this yet.

Fumaric acid (COOH.CH : CH.COOH).—This is the isomer of maleic acid, the latter being the cis-form, whereas fumaric acid is the trans-form (Fig. 1). Krebs (1938) states that fumarate and molecular O$_2$ are equally good as H-acceptors if glucose is the donor. In this case glucose is oxidized and fumarate reduced to succinic acid. Adrenaline acts similarly on glucose (see further on). Krebs assumes that the total H of glucose passes through fumaric acid when glucose is dehydrogenated. Fumaric acid increases respiration (Szent-Györgyi, 1937).

Malic acid (COOH.CHOH.CH$_2$.COOH).—This acid yields oxalacetic acid and succinic acid on oxidation and reduction respectively (Fig. 1). Malic acid is a very important link in the chain of reactions in respiration. It should not be confused with maleic or malonic acids. In nature *l*-malic acid is found, and its presence in wheat has been reported (Nelson and Hasselbring, 1931). Grewe and Child (1930) used the acid in place of tartrate in cake.

Alloxan.—Szent-Györgyi (1938a) believes that flavines may possibly be placed between malic and fumaric acid in the scheme of respiration. The main component of flavines is alloxazine, which combines with a sugar radicle to form lactoflavine (riboflavin, vitamin B$_2$), a widely distributed substance in nature. An important part of the

[1] Sullivan *et al.* (Cereal Chem. 17: 507–528, 1940) make a similar statement.

alloxazine molecule is alloxan, and H-acceptor, which can be reduced to dialuric acid.

Since alloxan inactivates papain (Purr, 1935), oxidizing the SH group (Dixon, 1939), it should prove useful as a bread improver. It has already been used as such, but fairly large quantities are necessary (Bungenberg de Jong, 1939; Jørgensen, 1938). According to the latter, dialuric acid—the reduced form—proved even better than alloxan According to Belgian patent 415,652 (1936) 0.002% is the usual amount necessary for improving a dough.

The results obtained with these four products were as shown in the tables. Table I shows that succinic acid and its oxidation product, fumaric acid, did not oxidize glutathione directly.

In the baking test (Table IV) alloxan, succinic, and fumaric acids showed an improvement, whereas malic acid did not. Only in the first case does the effect seem to be due to the inhibition of papain action (Table III, 11), although this was only partial, because the alloxan was not completely dissolved. Neither of the three organic acids inhibited the action of the papain preparation alone nor in the presence of glutathione (Table II, 15–17; Table III, 12–17).

Farinograph tests were made using 85% flour, 15% commercial wheat germ, and 0.1% succinic, fumaric, or malonic acids, omitting malic acid. After mixing and an hour's rest, the unchanged strong increase in mobility showed that none of the acids had any beneficia effect whatever, even in the presence of flour constituents. Even if the germ did contain succinodehydrase, the poisoning of this enzyme with malonic acid did not improve the physical condition of the dough.

Factors Influencing Respiration

Numerous substances are known to influence respiration in some way or other. Four of these were tested.

Adrenaline (epinephrine).—This is the first hormone to be synthesized. It exists in blood in a concentration of 1 in 1 billion, and is also found in the suprarenal glands. Adrenaline has been classified among the oxytrope catalysts; *i.e.*, it needs no intermediary catalysts to take up oxygen. Adrenaline is an unstable weak base, a strong poison, and a strong reducing agent. It is readily oxidized by oxygen, yielding a brown-colored substance; a ketone named adrenalone is produced (Sherman, 1928). Adrenochrome, another oxidation product, has also been obtained from adrenaline. Sherman also states that the rate of oxidation of glucose is increased by adrenaline. It acts quickly but only for a short time, whereas thyroxin acts more slowly but for a longer time. Because of this fact, thyroxin might be found to give

better results as a dough improver than adrenaline, although both are out of the question for practical use.[2]

Adrenaline is sparingly soluble in cold water. In warm water it oxidizes and is then fairly soluble. It can be dissolved in water at 60°C. If heating is short the solution is red; on prolonged heating it becomes yellow. Neither of these solutions improved loaf volume alone (Table IV, 8, 9). In the presence of a trace of copper a better oxidation of the dough was obtained, and in one test even a badly over-treated loaf was produced. The oven spring was good.

These results can be explained by the action on glutathione, as shown by results in Table III (23-26). Here too it was found that a mixture of oxidized adrenaline with a trace of copper was better than each separately. Copper sulfate alone also produced a partial oxidation. The importance of copper in dough oxidation has been emphasized by Bungenberg de Jong and Klaar (1939) and by Berliner (1939).

Ascorbic acid ($C_6H_8O_6$).—This acid (vitamin C) plays an important part in Szent-Györgyi's (1938a) system of oxidation of plant tissues, together with succinic acid and quinone. The oxidation of ascorbic acid leads to dehydroascorbic acid. Bezssonoff and Woloszyn (1938) found a form intermediate between the two. On addition of iodine at pH below 4 the first oxidation stage is formed, at high pH the dehydro form occurs (Wurmser, 1935). In tissues rapid reduction of the dehydro acid is brought about by glutathione (Borsook *et al.*, 1937; Kohman and Sanborn, 1937). Since its discovery as a bread improver by Jørgensen (1935) several authors have reported favorably on its efficiency as such.

Alpha-amylase was shown by Rupp and Bailey (1937) to alter the physical properties of gluten. Part of the beneficial action of dehydro-ascorbic acid in dough may be due to the fact that this acid inhibits alpha-amylase action (Euler *et al.*, 1934; Hanes, 1935; Weidenhagen, 1936). Melville and Shattock (1938) demonstrated that the dehydro acid is a more efficient improver than ascorbic acid itself and that the improving action is due entirely to the oxidation product. They also found that there exists in flour a mechanism whereby this oxidation takes place. The oxidation of ascorbic acid can, however, also be rapidly brought about by a peroxidase system (Fischer, 1937). Pulkki and Puutula (1938) determined the ascorbic acid content of germinating wheat.

Hopkins and Morgan (1936) stated that in the absence of ascorbic acid oxidase, dehydroascorbic acid is reduced five times slower than in its presence. By titration Ziegler (1940) found that dehydroascorbic

[2] Adrenaline strongly inhibits the autoxidation of unsaturated fatty acids (Oppenheimer and Stern, 1939, p. 121).

acid alone oxidized glutathione only about half as rapidly as bromate did.   Melville and Shattock (1938) state that the action of dehydro-ascorbic acid, on a weight-for-weight basis, was equal to bromate as an improver.   This difference may be explained by the presence of the oxidase in flour.   We have, however, always found dehydroascorbic acid the weaker of the two in its action.   The titration curves (Fig. 5) show that, in the absence of flour constituents and especially of as-corbic acid oxidase, dehydroascorbic acid oxidizes glutathione slower than the same amount of bromate does; *i.e.*, it is reduced much slower than bromate is.   The action is shown clearly in the turbidity tests (Table II, 11, 12).   Ascorbic acid, being a reducing agent, could not be expected to inhibit papain action in the absence of an oxidase. Even 30 mg. of dehydroascorbic acid did not inhibit as strongly as did 5 mg. of bromate.
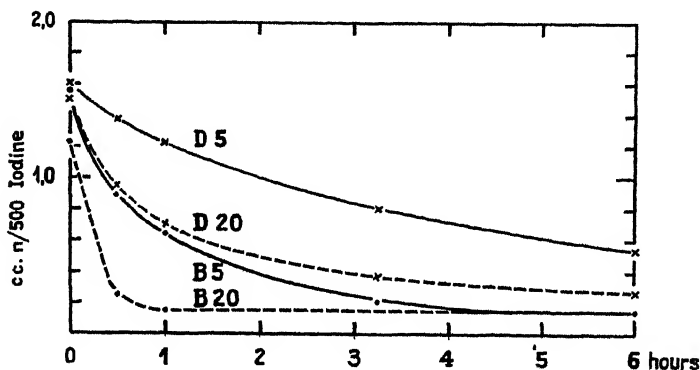
Fig. 5.   Rate of oxidation of glutathione by potassium bromate (B) and dehydroascorbic acid (D). In all cases 1 mg. of GSH in 10 cc. of distilled water was treated at room temperature with 5 mg. and 20 mg. of the oxidizing agents.

Maleic acid.—This compound and fumaric acid are isomers (Fig. 1). Maleic is the stronger acid.   It does not enter into respiration itself; in fact it was shown by Thunberg to be a strong poison of respiration (Szent-Györgyi, 1938), interacting with succinodehydrogenase and thus stopping any further O_2 uptake (Morgan and Friedmann, 1938a). The latter (1938, 1938a) found that maleic acid can combine with glutathione to form a thiosuccinic acid derivative inhibiting the cata-lyzing action on enzymes by the SH compound.   They showed that the interaction does not go on to completion.   Maleic acid is stated by Triebold *et al.* (1933) to be a useful agent against rancidity, *i.e.*, against a certain type of oxidation of fat.

As previously reported (Ziegler, 1940) and again shown in Table I (8), maleic acid did not oxidize glutathione within an hour.   Added

to a dough it had no good effect on oven spring and a decidedly deleterious effect on crumb texture.[3] The turbidity test, however, showed a marked inhibition by maleic acid, of the glutathione activation of papain action, although the effect is not quite so strong as with bromate (Table II, 21–24). The acid also had an inhibiting effect on the papain preparation itself (Table II, 18), in agreement with Morgan and Friedmann (1938a).

Malonic acid (COOH.CH$_2$.COOH).—Minute quantities of malonic acid poisoned respiration almost as cyanide does (Szent-Györgyi, 1937). This is because the oxidation of succinic to fumaric acid is inhibited (Fig. 1). Either pyrophosphate or malonic acid specifically poisons the enzyme succinodehydrase. This enzyme can reduce papain and cystine (Bersin, 1933, 1935). It occurs in all animal tissues, yeast, and bacteria, although some tissues are said to be deficient (Oppenheimer and Stern, 1939). By the use of malonic acid it might then be possible to inhibit the reduction of papain in dough, *i.e.*, to make it inactive, which should result in improved baking quality. A Berlin firm at one time put a malonic acid product called "Matagen" on the market and Neumann (1929), without giving the actual analysis, mentions that 0.05%–0.1% Matagen had a decided improving effect in a dough. It has the advantage of being volatile, so that no foreign substance is left in the bread. Traces of this acid have been found in wheat itself (Nelson and Hasselbring, 1931).

The turbidity test (Tables II, 19; Table III, 18, 19) showed no action against the reduction of papain, in spite of the fact that in all probability in our tests no succinodehydrase, which would tend to reduce papain, was present. The effect claimed for malonic acid does not seem to be explained by an action on glutathione. Our baking tests did not reveal malonic acid as a very good improver (Table IV, 7). As already mentioned, the farinogram of a flour and germ dough was not improved by malonic acid addition.

Other Factors

Aneurin (*thiamin, vitamin B$_1$*).—This strongly alkaline, sulphur-containing vitamin is involved in the conversion of pyruvic acid to acetaldehyde and CO$_2$ (Williams, 1939), and is therefore important in alcoholic fermentation (Schultz, Atkin, and Frey, 1937). We are interested in aneurin as a redox substance; on oxidation thiochrome is formed, on reduction a dehydro compound (Lippman, 1936; Boulanger, 1938). The latter can again be oxidized to aneurin. Recently Schultz, Atkin, and Frey (1939a) have determined the vitamin B$_1$ content of wheat, flour, and bread.

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[3] Further baking tests confirmed the results.

TABLE IV

Effect of Organic Additions + Oxygen on Loaf Volume

(1 mg. organic additions and in two cases 0.05 mg. copper or ferro salt were added per 100 g. flour from a Canadian-Argentine wheat mixture. Doughs mixed in hand-driven iron mixer with addition of oxygen.)

No.		Volume in %	Improvement
1	Untreated	100	
2	Potassium bromate	113	*
3	Succinic acid	106	*
4	Fumaric acid	105	*
5	Malic acid(inactive form)	97	
6	Alloxan	110	*
7	Malonic acid	104	
8	Adrenaline (red)	100	
9	Adrenaline (yellow)	102	·
10	Adrenaline (yellow) + $CuSO_4$[1]	110	*
11	Adrenaline (yellow) + $FeSO_4$	100	
12	Aneurin (thiamin)	100	
13	Nicotinic acid amide	100	
14	Asparagine	100	
15	"Chloramin" (Heyden)[2]	97	

[1] 0.05 mg. $CuSO_4$ alone gave 106%.   [2] 2.5 mg. used.

Nicotinic acid amide ($C_5H_4N.CONH_2$).—This is the pyridine nucleus of codehydrase I (cozymase) and of codehydrase II and can be reversibly hydrogenated, taking up and giving off two H atoms, as was first shown by Warburg. The vitamin B factor held responsible for "black tongue" in dogs has been obtained in a pure state and identified with nicotinic acid amide (Elvehjem; reported by Haurowitz, 1938).

Asparagine.—This amino acid has been found to inhibit amylase action (Filipowitz, 1931) but to stimulate maltose fermentation (Schultz *et al.*, 1939), *i.e.*, just the opposite to what formaldehyde does. It occurs in the germ of many plants and was found to have a deleterious effect on baking quality (Willard and Swanson, 1913; Bull, 1937) although Sullivan *et al.* (1936) state that it has only a slight shortening effect.

"Chloramin" (*p-sodium toluolsulphochloramide*).—This well-known disinfectant was found to inhibit the attack of papain on gelatin by Jørgensen (1938), thus confirming the findings of Elion.

Our tests with the foregoing substances show the following results:

Aneurin and nicotinic acid amide did not inhibit glutathione-activated proteolysis (Table I, 10; Table III, 27, 28).

Asparagine was included to see whether its deleterious effect can

be brought into relation with proteolysis. Results in Table III show that it neither activated the papain (21, 30) nor did it decrease the glutathione activation (22, 29).

As was expected as a result of Elion's and Jørgensen's work, "chloramin" (Heyden) decreased the turbidity which the papain preparation would have produced with or without added glutathione, although large amounts were needed, as mentioned by Jørgensen (Table III, 31–32).

The gassing power of the nicotinic acid amide and asparagine doughs was rather weak; that of the "chloramin" dough was poor. The loaf volume (Table IV) revealed nothing noteworthy. The texture was fair in all four cases.

Discussion

The improvements in baking quality obtained with oxygen are always less than that obtained with bromate, as was also shown by Freilich and Frey (1939). It was thought that a catalyst might be found among substances known to increase or to act as carriers in respiration, so that the oxygen could improve dough to a greater extent. This could not be demonstrated.[4] The results found have been summarized in Table V.

The iodine titration method, as well as the turbidity test used, have both shown that the effect of the following flour improvers can (at least partly) be explained by their oxidation of glutathione: bromate, iodate, persulphate, and dehydroascorbic acid. Chlorate alone did not react, which is further confirmation of the validity of the results obtained by these two methods. These same substances also inhibited the breakdown of gluten by a commercial papain preparation, seemingly in the absence of an activator. The results with papain, however, cannot be interpreted as proving that the oxidizing agents act directly on the pure enzyme, as the preparation may have contained an activator, although no heat-resistant activator could be detected. So far no published data have been found on work with *pure* papain.

The turbidity test could be developed as an excellent method, especially if a real nephelometer were used. It proves useful for rapidly controlling the proteolytic action of various papain preparations, as these can be allowed to work on exactly the same substrate (gluten flour, which can be stored for several years) and under the same conditions.

Baker and Mize (1939) contend that in the absence of both yeast

[4] Weil-Malherbe (referred to by Oppenheimer and Stern, 1939) found that succinic acid, when added to animal tissues, behaves differently from that actually found in these tissues. The same held for fumaric acid and malic acid.

TABLE V

RELATIONSHIP BETWEEN LOAF VOLUME INCREASE AND INHIBITION OF
PROTEOLYSIS FOR SOME ORGANIC AND INORGANIC SUBSTANCES

Oxidation of glutathione and/or inhibition of proteolytic action		
Negative		Positive
Improvement in loaf volume		
None	Slight	Definite
INORGANIC OXIDIZING AGENTS		
Chlorate		Bromate
		Iodate
		Persulphate
		Metaranadate
FACTORS INVOLVED IN ANIMAL RESPIRATION		
Malic acid	Succinic acid	Alloxan
(inactive)	Fumaric acid	
FACTORS INFLUENCING RESPIRATION		
	Malonic acid	Adrenaline ($+Cu^{++}$)
		Dehydroascorbic acid
		Maleic acid [1]
FURTHER FACTORS		
Aneurin		
Asparagin		

[1] Poor oven spring and coarse texture.

fermentation and mechanical action, bromate has little apparent effect. The function of these two factors is probably the better distribution of the oxidizing agent in the dough, which enables it to reach the glutathione and perhaps the proteolytic enzyme more easily. Neither fermentation nor mechanical treatment is necessary for the inactivation of proteolysis itself.

Summary

The results herein reported are in line with the theory of Jørgensen (1935) which is proving a useful basis for the explanation of the mechanism of dough improvement due to the inhibiting action of oxidizing agents on proteolysis.

Factors which increase respiration (succinic and fumaric acid and adrenaline) had a certain improving action on baking quality, although only adrenaline, in the presence of a trace of copper, actually inhibited proteolysis.

Of the two factors studied which poison respiration (malonic and maleic acid), malonic had no effect on proteolysis. Maleic acid did inhibit glutathione activation even in strong acid medium. Neither class of factors that increase or poison respiration seems to have a direct effect as such on baking quality.

The rate of oxidation of glutathione by dehydroascorbic acid in the absence of oxidase was compared to that by bromate and shown to be much slower.

The turbidity method used proved useful, rapid, and simple. It can be used to determine which agents improve dough quality due to their action on glutathione.

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THE DETERMINATION OF TOTAL BETA–AMYLASE IN BARLEY AND BARLEY MALT [1]

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Recent contemporary workers propose that the total saccharifying power in barley may be estimated by digesting the ground barley with papain. Myrbäck and Myrbäck (1933, 1936) attribute the increase in beta-amylase activity during germination to the proteolytic release of insoluble amylase bound to the protein molecule.

Chrzaszcz and Janiki (1933, 1936) hypothesize that certain " eleuto-substances " are formed during germination that nullify or counteract the inhibitory effect of a " sisto-substance " on beta-amylase activity.

Hills and Bailey (1938) concur with Myrbäck and Myrbäck and further conclude that the difference in the apparent increase in total beta-amylase during germination as measured by the rate of maltose production is in reality due to the increase in alpha-amylase activity.

Chrzaszcz and Swiatkowska (1937) in latter investigations with hydrogen sulfide and papain conclude that hydrogen sulfide in conjunction with papain liberates a strongly bound component of beta-amylase that is not liberated by papain alone. Sallans and Anderson (1938) are not in complete agreement with this view, and present considerable data to support the opinion that Chrzaszcz and others used a grade of papain of inferior strength that was simply activated by hydrogen sulfide. This reasoning seems appropriate as it was stated that the papain used in their experiment was 1 : 100 in strength. In their recent study of the hydrogen sulfide-papain procedure, Myrbäck and Örtenblad (1939) conclude that the results of Chrzaszcz and others were distorted through amylase poisoning, probably by water containing copper salts.

It has not as yet been conclusively shown that the ultimate amount of total saccharifying activity has been estimated by any of the recent procedures employed. There is no evidence in the data of Hills and Bailey (1938) that steeping, germinating, and rootlet losses were considered when the total saccharifying power found in the barley was compared with the total saccharifying power found in the corresponding green malt. Chrzaszcz and Swiatkowska (1937) showed a maximum amount of saccharifying activity estimated under certain specific condi-

tions. They did not prove, however, that the entire amount of beta-amylase had been released and estimated.

Analyses for total saccharifying activity of barleys and green malts of the barleys, malted in the Grain Research Laboratory, Agricultural Marketing Service, Washington, D. C., in all cases indicated the presence of more beta-amylase in the green malt than in the barley, even when expressed in terms of the barley. These results may be interpreted as indicating one of two probabilities, that the analytical method employed did not estimate the entire amount of saccharifying activity in the barley, or that other enzymatic activity in the green malt such as alpha-amylase is erroneously estimated as beta-amylase additional to the total amount found in the barley.

In the earlier phases of this research, many barley and malt extracts were treated with papain and hydrogen sulfide at variable concentrations, times, temperatures, and hydrogen-ion concentrations, with little success. In every instance the result obtained by the use of papain alone was higher than those obtained by any papain-hydrogen sulfide combination.

The experiments discussed in this paper were performed for the purpose of developing a procedure providing optimum conditions of activation, hydrogen-ion concentration, time, and temperature for the extraction of the total beta-amylase in barley and malt, and indicating whether or not the last vestige can be released and estimated.

Barley Sample

A bulk sample of sound barley was finely ground and sieved to remove large particles and bran coat, in order to minimize variability within the sample. That portion passing through a No. 35 U. S. standard sieve was used in all subsequent experiments, and is designated as experimental sample No. 1. The papain was also sieved to remove lumps that might interfere with uniform mixing. A teaspoonful of 60-mesh alundum was added to all mixtures to prevent the papain from balling and to aid in obtaining a uniform paste from the mixture, before adding the entire amount of the extraction solution.

Papain

The selection of a satisfactory papain is most important, as commercial grades vary considerably in enzymatic strength. Three commercial samples, represented as 1 : 200 in strength, were selected and analyzed according to the method of Balls and Hoover (1937). One percent solutions of these samples, with and without cysteine, were used for the extraction of experimental barley sample No. 1. Diastatic power of the extracts was determined and the values are recorded in Table I.

Papain No. 1 and No. 2 are about standard strength for commercial grades. Papain No. 3 is unsatisfactory, as is evident from the results shown in the table. The use of cysteine in these experiments will be explained under " activation."

TABLE I

COMPARISON OF THE RELATIVE ACTIVITY OF THREE COMMERCIAL PAPAINS OF VEGETABLE ORIGIN

Experimental Sample No. 1

200 cc. of 5% extract for 20 hours at 20°C.

Papain			Activity units of Klim[1]	Cysteine HCl (neutralized)	Total saccharifying power	
No.	Identity	Amount			A.S.B.C. method	FeCy method
		g.		g.	°L.	°L.
1	Unknown	2	0.38	0	87.4	87.8
		2		1	82.8	85.4
		2		2	82.8	86.1
2	Ceylon	2	0.41	0	86.2	88.9
		2		1	84.6	88.3
		2		2	82.8	89.4
3	Mexican	2	0.06	0	66.1	71.5
		2		1	69.1	74.6
		2		2	72.1	76.5

[1] According to the method of Balls and Hoover (1937).

Time and Temperature for Extraction

Extractions were made at variable concentrations of barley and of papain, at variable temperatures and periods of time with and without toluene. The results of these experiments are shown in Table II.

An examination of the data in Table II shows: that 24 hours or thereabout is required to release the maximum amount of beta-amylase when papain alone is used; that no more beta-amylase can be obtained by prolonging the extraction time to 48 hours; and that a one percent solution of papain is sufficient for this purpose. It also appears that the addition of toluene to preserve the extract is not necessary if extractions are made at 20°C. Some extractions (not reported in Table II), made at room temperatures of 25°C. to 30°C. for 20 to 24 hours, showed slight evidence of fermentation when toluene was not used, and that the diastatic values were slightly lower than those of the preserved solutions. Briefly, it appears that the maximum yield can be obtained by extracting with one percent solutions of papain for 24 hours at 20°C. There is some evidence in the small amount of data shown that the same extraction could be accomplished in 3 hours at 40°C.

TABLE II

THE EFFECT OF VARIABLE CONCENTRATIONS OF BARLEY AND PAPAIN AT
VARIABLE TIME AND TEMPERATURE

Barley Sample No. 1

Test No.	Barley	Papain	Water	Tem-perature	Time	Toluene	Dia-static power
	g.	*g.*	*cc.*	*°C.*	*hrs.*	*cc.*	*°L.*
1	25	3	150	20	24	4	89.2
2	25	4	200	20	24	4	88.9
3	10	2	200	20	24	4	90.5
4	10	2	200	20	24	–	89.5
5	10	2	200	20	48	–	88.2
6	10	2	200	20	48	4	87.6
7	10	4	200	20	24	–	88.0
8	10	3	200	20	24	–	88.6
9	10	2	200	20	24	–	89.5
10	10	1	200	20	24	–	87.5
11	10	0.5	200	20	24	–	85.1
12	10	0	200	20	24	–	25.6
13	10	4	200	20	3	–	85.3
14	10	3	200	20	3	–	83.8
15	10	2	200	20	3	–	82.9
16	10	1	200	20	3	–	78.3
17	10	0.5	200	20	3	–	72.9
18	10	0	200	20	3	–	24.0
19	10	4	200	40	3	–	86.5
20	10	2	200	40	3	–	89.4
21	10	1	200	40	3	–	80.8
22	10	0.5	200	40	3	–	77.9

Five percent extracts of barley and malt were made in all succeeding experiments, for convenience in a comparison of diastatic activity values by an accepted standard method.

Activation

It is generally known that the activity of papain can be accelerated by activation with hydrogen sulfide, hydrocyanic acid, cysteine, or glutathione. The initial trial experiments with hydrogen sulfide-papain combinations in this research confirmed the general opinion that it is an unsatisfactory agent for use when applied to the substrates of barley and malt. The hazard involved discourages the use of hydrocyanic acid for routine analytical purposes. Cysteine is an excellent activator of papain if complete oxidation of the sulfhydryl group is not effected during the course of activation. Glutathione does not possess any advantage over cysteine and it is too expensive for practical use; furthermore, cysteine is a constituent of the glutathione molecule. The use of cysteine as the activator facilitates the preparation of the extraction solution.

Very stable, although strongly acid, solutions of cysteine may be prepared with c. p. cysteine hydrochloride. When this solution is neutralized, as required, its stability gradually diminishes. Prepared stock solutions of cysteine hydrochloride deteriorated overnight, when neutralized, with the subsequent formation of cystine, which has no activating effect on papain. The stability of acid and neutral solutions of cysteine is shown in Table III.

TABLE III

STABILITY OF ONE PERCENT SOLUTIONS OF CYSTEINE-HCl AND CYSTEINE-HCl, NEUTRALIZED

Solution No.	Days standing before testing	Solution concentration in parts					
		1:100		1:1000		1:10000	
		Time[1]	Color[2]	Time[1]	Color[2]	Time[1]	Color[2]
		min.		min.		min.	
1	0	6	Deep purple	6	Pink	6	Yellow
		17	Green brown	17	Yellow	–	—
		24	Yellow	˙–	—	–	—
2	1	7	Deep purple	7	Pink	7	Yellow
		16	Green brown	16	Yellow	–	—
		25	Yellow	–	—	–	—
3	3	6	Deep purple	6	Pink	2	Yellow
		15	Light brown	15	Yellow	–	—
		22	Yellow	–	—	–	—
4	20	5	Deep purple	5	Pink	–	—
		15	Light brown	15	Yellow	–	—
		21	Yellow	–	—	–	—
1a[3]	1	12	Dark brown	12	Yellow	–	—
		20	Yellow	–	—	–	—
2a[3]	3	12	Light brown	2	Yellow	–	—
		17	Yellow	–	—	–	—

[1] Spot plate test, reading after adding nitroprusside reagent.
[2] Cysteine is completely oxidized to cystine when the characteristic yellow color of the mixed reagents is attained.
[3] Solutions 1 and 2, respectively, neutralized.

It is important to know the extent of cysteine oxidation during the extraction and filtration period, in order to ascertain the minimum amount of cysteine necessary to insure complete activation during the entire extraction period.

Cysteine may be detected in the presence of cystine by the nitroprusside reaction. A simple qualitative test to check the stability of the — SH group in extracts after the extract has been filtered and diluted, is to treat about 10 drops of the test solution on a spot plate with 5 to 10 drops of ammonium hydroxide (the solution must be alkaline) and 3 to 5 drops of sodium nitroprusside. Cysteine develops a purple color that varies in intensity with the concentration of cysteine present and that slowly fades as the cysteine is oxidized to cystine. One part of cysteine in 10,000 gives a distinct color reaction. It was observed that

diluted extracts 1:10, tested for cysteine stability, gave lower beta-amylase values when the characteristic color faded completely in less than 10 minutes.

In all experiments the cysteine hydrochloride was neutralized before using. Solutions 1a and 2a, Table III, are the neutralized solutions of 1 and of 2, respectively, that have been allowed to stand for 1 and 3 days respectively before testing by the nitroprusside method.

Extracts of experimental barley sample No. 1, with variable amounts of papain and cysteine, with and without citrate buffer, were tested for cysteine stability and diastatic power. The results are shown in Table IV. Test No. 6 gave a weak nitroprusside reaction and a correspond-

TABLE IV

THE EFFECT OF ACTIVATION OF PAPAIN WITH CYSTEINE IN THE EXTRACTION
SOLUTION, 3 HOURS AT 20°C.

Test No.	Papain	Cysteine HCl	Citrate buffer	pH of extract	Nitro-prusside reaction	Diastatic power
	g.	*g.*				°*L.*
1	2.0	2.0	0	5.8	Deep purple	88.6
2	1.0	1.0	0	5.8	"	89.4
3	0.8	0.8	0	5.8	"	91.2
4	0.6	0.6	0	5.8	Purple	92.3
5	0.4	0.4	0	5.8	"	91.4
6	0.2	0.2	0	5.8	Faint purple	80.5
7	2.0	2.0	0.02M	5.0	Deep purple	82.5
8	1.0	1.0	0.02M	5.0	"	86.2
9	0.8	0.8	0.02M	5.0	"	87.1
10	0.6	0.6	0.02M	5.0	Purple	85.1
11	0.4	0.4	0.02M	5.0	"	86.0
12	0.2	0.2	0.02M	5.0	"	86.4
13	2.0	0.5	0	5.8	"	84.9
14	1.0	0.5	0	5.8	"	87.8
15	0.8	0.5	0	5.8	"	86.8
16	0.6	0.5	0	5.8	"	87.3
17	0.4	0.5	0	5.8	"	87.1
18	0.2	0.5	0	5.8	"	86.8

ingly low diastatic power. The optimum ratios of papain and cysteine hydrochloride appear to be between 0.6 and 0.8 g. each for an extraction of 10 g. of barley.

Effect of the Hydrogen-Ion Concentration

The effect of buffering the extraction solution for optimum activity of papain and cysteine was studied. Papain is most active in isoelectric solutions with optimum activity occurring at the isoelectric point of the protein substrate. Since there is no common isoelectric point for the proteins of barley, any arbitrarily chosen isoelectric point within the

isoelectric zone of the amylase-binding proteins should satisfy the conditions for optimum activity of papain.

Murray (1933) found that citrate buffers were superior to acetate and phthalate buffers for pH control of substrates subjected to the action of papain. Other conclusions were: that the citrate effect was a salt effect, extending the pH zone of activity, and that the activating effect of citrate was additive to that of other activators. It is known that citrate salts fix heavy metals. The presence of copper salts, for instance, in the steep waters would result in the inhibition of enzymatic activity. Weidenhagen (1936) found that vitamin C (ascorbic acid), even in minute quantities, reduced amylase activity, but that citric acid acted to a certain degree as an inhibitor to vitamin C.

In view of these useful properties of citrate salts for this type of enzymatic reaction, the importance of employing a citrate buffer, if for nothing more than as a precautionary measure, cannot be ignored. Certainly no ill effects attributable to the use of a citrate buffer could be observed in any of these experiments.

Combinations of papain-cysteine-treated extracts in the presence of citrate buffer at various pH and molarity were analyzed. Because of the difficulty of preparing citrate buffers at pH 7.0 phosphate buffer solutions were substituted for extractions at that hydrogen-ion concentration. The results are given in Table V, in which extracts without buffers are compared with the buffered extracts. It appears that opti-

TABLE V

Effect of the Hydrogen-Ion Concentration on the Estimation of Total Saccharifying Power in Barley and Malt Extracts

Experimental Sample No. 1

10 g. extracted with 200 cc. solution for 3 hours at 20°C. Extract Nos. 1, 2, and 3 contain 600 mg. of papain and 600 mg. of cysteine-HCl. Extract Nos. 4, 5, and 6 contain 800 mg. of papain and 800 mg. of cysteine-HCl.

Extract No.		Buffer solutions (extract)								
	Molarity	Citrate pH 5.0		Citrate pH 6.0		Citrate pH 6.5		Phosphate pH 7.0		None	
		D.P.[1]	pH	D.P.[1]	pH	D.P.[1]	pH	D.P.[1]	pH	D.P.[1]	pH
1	0.02	°L. 85.1	5.0	°L. 86.4	5.6	°L. 89.7	6.2	°L. 84.7	6.7	°L. 89.5	5.8
2	0.05	87.4	5.0	89.2	5.8	90.5	6.3	88.4	6.8	—	—
3	0.10	—	—	89.2	5.9	90.1	6.4	89.4	6.9	—	—
4	0.02	87.1	5.0	86.8	5.6	90.1	6.2	85.5	6.7	91.2	5.8
5	0.05	—	—	87.1	5.8	90.8	6.3	88.5	6.8	—	—
6	0.10	—	—	86.4	5.9	91.9	6.4	90.5	6.9	—	—

[1] Diastatic power, degrees Lintner.

mum results are obtained with $0.05M$ citrate buffer concentration at pH 6.5, although differences of less than 3° Lintner are really not significant.   The use of a citrate buffer is precautionary, as has been explained.

Oxidation and Reduction Studies with Phenylhydrazine

Phenylhydrazine is regarded by Bergmann (1936) and Bergmann and Ross (1935) as an activator of natural papain.   It is presumed by Balls and Hoover (1937) that this activation is due to the reduction of the natural activator, analogous to its reduction of methylene blue. A few miscellaneous trial experiments were performed to observe the influence of phenylhydrazine in the presence of the various reagents. These results are shown in Table VI.   It is apparent that phenylhydra-

TABLE VI

THE INFLUENCE OF PHENYLHYDRAZINE ON THE REAGENTS USED IN THE
EXTRACT FOR DETERMINING TOTAL SACCHARIFYING POWER

Experimental Sample No. 1

200 cc. of 5% extract for 20 hours at 20°C.

Ex-tract No.	Pa-pain [1]	Cysteine HCl [2]	Solution concentration			Color reactions		D.P.[6]
			Citrate buffer [3]	Solution pH	Phenyl-hydrazine [4]	Methylene blue [5]	Nitro prusside	
	mg.	*mg.*						
1	600	600	0	—	0	{Faint green-ish blue	Purple	86.6
2	600	600	.05 M	6.3	0		"	87.4
3	600	600	0	—	.05 M	{Light blue	"	86.2
4	600	600	.05 M	6.3	.05 M	restored	."	88.5
5	600	0	0	—	0	{Blue	—	86.3
6	600	0	.05 M	6.3	0	Blue	—	89.2
7	600	0	0	—	.05 M	{Colorless	—	5.2
8	600	0	.05 M	6.3	.05 M	Colorless	—	35.7

[1] Dry powder added to ground barley.
[2] Neutralized with n-NaOH to pH 6.5±.
[3] Final molarity of buffer in the extract.
[4] Final molarity of phenylhydrazine in the extract.
[5] 0.25 cc. of saturated solution of methylene-blue added.   Color noted at the beginning and end of the extraction period.
[6] Diastatic power, degrees Lintner.

zine does not materially assist cysteine in the reduction of papain.   The activating effect of citrate buffer is considerably impaired when phenylhydrazine is present.   There appeared to be an actual inhibition of diastatic activity when phenylhydrazine was used in the absence of cysteine and buffer solution.   (The free diastatic power of the barley sample was 25.6° Lintner.)   It is assumed from the information accumulated from these experiments that sufficient cysteine was present to reduce completely the papain and carry the reaction to completion.

Development of a Procedure

The data accumulated in all of the preceding experiments seem to justify the assumption that (1) as much, if not more, saccharifying activity can be obtained from ungerminated barley by the use of 0.6 g. to 0.8 g. of cysteine and equal amounts of papain in three hours' extraction as can be obtained with 2 g. of papain alone in 24 hours' extraction, (2) that citrate buffer, 0.05 molar concentration, pH 6.4 to pH 6.7, may be used as a precautionary measure to prevent any possible inhibition due to heavy metals or vitamin C, without affecting the results, (3) that temperatures higher than 20°C. do not give a higher yield, and (4) that the addition of toluene as a preservative is not necessary for the short-time extraction. These indications would suggest the following short method for the determination of total diastatic power in ungerminated barley.

Reagents

Citrate buffer solution: 0.2M, pH 6.4–6.6.

> *Solution a:* Dissolve 82.016 g. of c.p. citric acid ($C_6H_8O_7.H_2O$) in 400 cc. of carbonate-free 1.0N NaOH, add 1 cc. of toluene for preserving, and dilute to 1 liter. The pH of this solution should be 4.96.

> *Solution b:* Mix 530 cc. of solution *a* with 470 cc. of 0.2N NaOH. The pH of this solution should be about 6.6.

Phosphate buffer solution: 0.1M, pH 8.3. Dissolve 14.2 g. of c.p. anhydrous Na_2HPO_4 in CO_2-free distilled H_2O, add 1 cc. of toluene, and make up to 1 liter.

Cysteine-HCl solution: Dissolve 18.75 g. of c.p. cysteine-HCl in distilled H_2O and make up to 500 cc. Twenty cubic centimeters of this solution should require 4.5 cc. of 1.0N NaOH for neutralization.

Procedure

For barley: Pipette 20 cc. of cysteine-HCl solution into a 200-cc. volumetric flask and neutralize with 4.5 cc. of 1.0N NaOH, add 50 cc. of citrate buffer solution *b* and make up to 200 cc. with distilled H_2O. Test this solution with brom-thymol blue on a spot plate. The pH should fall within the range of 6.4 to 6.7.

Weigh out 10 g. of finely ground barley into a 250-cc. Erlenmeyer flask. To this, add 750 mg. of standard-strength papain and a teaspoonful (approximately 15 g.) of 60-mesh alundum. To this mixture add a small portion of the buffered cysteine solution to obtain a pasty consistency. Swirl the flask until all lumps are broken up. Add the remainder of the extraction solution, stopper the flask, and immerse in a water bath at 20°C. This extract should be agitated at intervals of 15 to 20 minutes. After 2½ hours, filter the extract through 18 ½ cm. S and S fluted filter paper. Cover the funnel with a watch glass to arrest atmospheric oxidation and evaporation. Reject the first 50 cc. of the

filtrate. If it is preferred to extract 25 g. of barley, the same ratio of reagents applies. Pipette 20 cc. of the extract (or 10 cc. if the diastatic power exceeds 135° Lintner) into a 100-cc. volumetric flask and dilute to 100 cc. At this stage proceed according to the method of Anderson and Sallans (1937) as outlined by Laufer, Schwarz, and Laufer (1938) for the determination of diastatic power by the ferricyanide method.

The procedure for green malts or kilned malts is the same unless it is desired to estimate the saccharifying activity of beta-amylase only. In this case the Ohlsson (1926, 1930) acidification technique for differential inactivation of alpha-amylase is employed. Ohlsson's method is not quantitative but may be used in studies pertaining to experimental malting. Otherwise the diastatic-power values of green malt represent the combined activities of alpha-amylase and beta-amylase. If a differential estimation is desired proceed in the same manner as in the analysis of barley up to the filtration of the extract. Pipette three aliquots of 20 cc. or 10 cc. (as the case may require) into a 100-cc. volumetric flask and into two 100-cc. Erlenmeyer flasks. Immerse the volumetric flask in an ice-water bath, using lead rings so that the flask may be almost entirely immersed. If feasible place the ice-water bath within the cooling compartment of a refrigerator; otherwise the bath must be placed in the coldest available spot within the refrigerator.

Predetermine electrometrically, on one of the aliquots, the amount of 0.1N HCl necessary to bring the extract to pH 3.3. The progress of this titration may be checked by titrating incrementally and testing the solution on a spot plate with brom-phenol blue as pH 3.3 is approached, to avoid the necessity of several electrometric determinations. When the temperature of the extract in the ice-water bath is reduced to 0°–4°C., add the predetermined amount of 0.1N HCl to the test solution and return the bath with the flask to the refrigeration compartment for 15 minutes.

To the third aliquot add the same amount of HCl as used in the other aliquots and determine electrometrically the amount of phosphate buffer solution necessary to restore the solution to pH 6.7. Remove the test solution from the refrigerator after 15 minutes of the acid treatment and add the predetermined amount of phosphate buffer solution required for neutralization. Dilute the solution to 100 cc. and proceed with the determination for diastatic power.

If desired, this diluted test solution may be tested on a spot plate for cysteine stability by treating 10 drops with 5 drops of 5% solution of sodium nitroprusside and 5 drops of 10% ammonium hydroxide. If the purple color of the mixture does not fade out completely in less

than 10 minutes, optimum activation of papain by the sulfhydryl group of cysteine has been maintained during the entire extraction period.

Application of the Method

The experiments thus far reported were made with one sample of barley of low diastatic power. Six varieties of barley ranging from low to high diastatic power were then selected and analyzed for total diastatic power by the old method (that is, digestion with a one percent solution of papain for 20 to 24 hours) and by the short procedure. The results are shown in Table VII. The Lintner values obtained by the modified

TABLE VII

COMPARISON OF TOTAL DIASTATIC POWER IN BARLEYS, DETERMINED BY THE
MODIFIED METHOD AND BY THE OLD METHOD

Sample No.	Variety	Old method		Modified method	
		D.P.[1]	pH of extract	D.P.[1]	pH of extract[2]
2	Atlas	79.8	5.5	84.0	6.5
3	Spartan	74.8	5.5	73.8	6.4
4	Velvet	109.6	5.6	110.0	6.5
5	Trebi	110.8	5.6	111.6	6.5
6	Oderbruker	157.6	5.5	164.0	6.4
7	Peatland	184.0	5.5	189.8	6.4

[1] Diastatic power in degrees Lintner.
[2] Extract buffered with citrate buffer, pH 6.7.

method are slightly higher than those of the old method. This is not definitely significant, as comparison is made with only a small number of samples. The results on green malts, however, are quite significant.

Nine samples of barley were malted until germination was completed according to recognized standard malting procedure. The green malt was removed from the germination cabinet and rapidly air-dried in a drying cabinet at 93°F. These samples of malt were analyzed for total diastatic power by the old method and by the short method. The green malts were analyzed for alpha-amylase activity by the method of Ehrnst, Yakish, and Olson (1939). Extracts for total beta-amylase of the green malt by the short method were treated for alpha-amylase destruction, with 0.1N HCl at 0°C. for 15 minutes and analyzed for diastatic power. The malting losses were determined during the course of malting. The original barley samples were analyzed for total saccharifying activity by the short method. The Atlas barleys, samples No. 11 and No. 12, were grown under unusual soil and climatic conditions, which may account for the exceptionally high diastatic values for this variety. The results are recorded in Table VIII.

TABLE VIII

Comparison of the Methods and Application of the Modified Method in the Analysis of Green Malts

Sample No.	Barley variety	Total saccharifying power, green malt, dry basis			Malting loss—steeping, germinating, and rootlet	Total saccharifying power, green malt corrected for malting losses to basis of barley			Total saccharifying power, barley	Variable [2]		Dextrinization values—modified Wohlgemuth method [3] green malt, dry basis
		Papain method [1]	Papain-cysteine method [2]	Papain-cysteine method [2] less alpha-amylase		Papain method [1]	Papain-cysteine method [2]	Papain-cysteine method [2] less alpha-amylase	Papain-cysteine method [2]	Green malt vs. barley, total saccharifying power	Green malt, total beta-amylase less alpha-amylase vs. barley	
		°L.	°L.	°L.	%	°L.	°L.	°L.	°L.	°L.	°L.	
8	Atlas	76.9	79.8	71.2	11.0	68.5	71.0	63.4	58.5	21.3	4.9	45.5
9	"	89.5	93.2	86.5	11.0	79.7	82.9	76.2	79.4	13.8	−3.2	48.8
10	"	75.5	81.7	76.5	11.3	67.0	72.5	66.9	63.9	17.8	3.0	49.8
11	"	168.6	180.6	130.0	10.7	151.2	161.6	116.3	122.5	58.1	−6.2	77.8
12	Velvet	166.0	180.0	146.5	10.2	149.1	161.0	131.6	124.0	56.0	7.6	82.1
13	"	259.6	272.0	201.1	11.0	222.1	244.0	179.0	180.7	91.3	−1.7	114.7
14	Wisconsin 38	277.8	288.4	220.0	10.0	250.0	259.6	198.0	203.1	85.3	−5.1	132.3
15	Manchurian	251.3	260.0	184.4	11.5	222.4	230.1	163.2	172.8	87.2	−9.6	119.5
16	Odessa	224.0	228.7	183.2	9.3	203.2	207.4	166.2	151.1	77.6	15.1	105.4

[1] Five % extracts with 1% solution of papain for 24 hours at 20°C.
[2] Modified method.
[3] Method of Ehrnst, Yakish, and Olson (1939).

There is a significantly higher saccharifying power by the short method than by the old method, determined in terms of degrees Lintner. The same extracts of green malt show considerably more diastatic power than the corresponding barleys after correction for malting losses. The extracts of green malt in which the alpha-amylase was destroyed, however, show nearly the same values, after correction for malting losses, as the barleys.

The difference in total diastatic power between barley and green malt, in which the alpha-amylase has not been destroyed, does not quantitatively correlate with the dextrinization values.

There is a relation, however, between the total and apparent increase in degrees Lintner, presumably produced by beta-amylase when the alpha-amylase has not been destroyed, and the dextrinizing power determined directly by the method of Ehrnst, Yakish and Olson (1939).
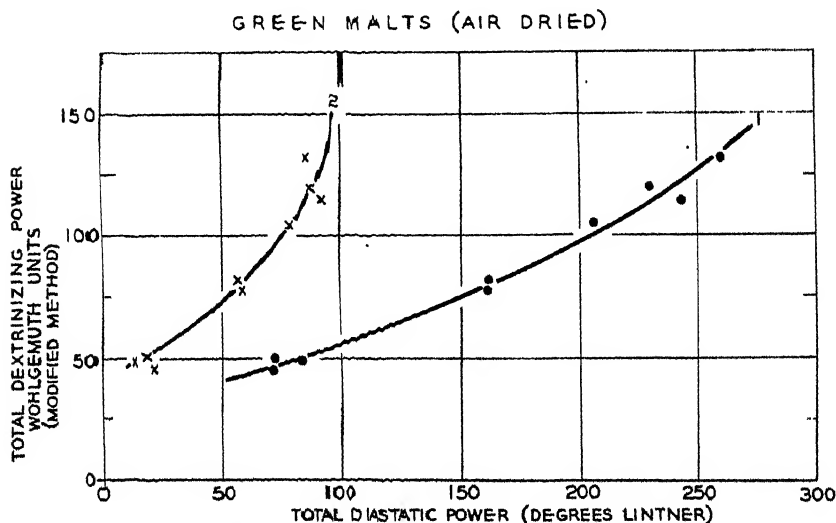

Fig. 1. Curve 1: relation of total diastatic power to dextrinizing power. Curve 2: apparent increase in saccharifying power of green malt over that of the corresponding barley due to the presence of alpha-amylase, compared with dextrinization values.

This is shown in Figure 1. These results indicate that the increase in maltose production in a starch substrate treated with malt extract over that treated with the corresponding barley extract is not entirely due to the additive alpha-amylase activity, and that the combined saccharifying power of alpha-amylase and beta-amylase exceeds the sum of their separate activities.

Freeman and Hopkins (1936) observed that the limit of hydrolysis with mixed amylases is only 10% to 12% greater than with single amylases of similar total activity, which suggested that at least half of the starch constitutes a substrate common to both enzymes.

It is demonstrated in Figure 1 that the effect of alpha-amylase on saccharifying activity becomes less apparent as the concentration of the amylases is increased. Similarly, Sandstedt, Kneen, and Blish (1939) found that the rate of dextrinization increased up to a certain point as the ratio of beta-amylase to alpha-amylase in the starch enzyme mixture increased.

Summary

The total beta-amylase of barley, green malt, and malt can be extracted in 3 hours with a papain-cysteine combination in the presence of a citrate buffer.

The saccharifying activity in barley and malt is more conveniently estimated by the proposed short-time extraction method than by extraction for 20 hours with papain alone.

The apparent increase in total beta-amylase in green malt over that of the corresponding barley appears to be due chiefly to the combined effects of alpha- and beta-amylase.

Papain used in the extraction mixture should be not less than 1:200 in strength.

The proposed method is useful for accurately predicting the total available beta-amylase in barley, and/or for gauging the efficiency of a malting procedure with respect to the production of beta-amylase from that barley.

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VALUE OF ASCORBIC ACID FROM VARIOUS SOURCES IN BAKING

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Breads including grapefruit juice in the formula are receiving consideration; thus the need for information regarding the value of added grapefruit juice from the viewpoints of nutritive values and baking properties becomes apparent. The vitamin C in grapefruit juice might enhance the nutritive value of bread. From available information (Cathcart, 1938) it also appears that grapefruit juice might be a valuable ingredient in bread from the standpoint of baking, possibly by acting as a valuable " dough conditioner." In order to obtain information on these two points, a preliminary investigation of grapefruit-juice bread was conducted.

General Discussion of the Problem

l-Ascorbic acid is a relatively labile compound under conditions which permit exposure to oxygen at elevated temperatures. Procedures employed in making bread permit considerable exposure to oxygen and require the use of elevated temperatures. In addition, flour contains an enzyme system which catalyzes the oxidation of *l*-ascorbic acid (Melville and Shattock, 1938). It follows that the *l*-ascorbic acid content of grapefruit-juice bread can not be calculated from the *l*-ascorbic acid content of the grapefruit juice added.

Jørgensen (1935) announced the discovery that *l*-ascorbic acid could act as a dough conditioner and that it has an effect similar to that of potassium bromate, an oxidizing agent. The action of *l*-ascorbic acid, like bromate, is said to involve an inhibition of the flour proteinases (Jørgensen, 1939). Melville and Shattock (1938) have shown that the enzyme ascorbic acid oxidase of flour catalyzes the oxidation of *l*-ascorbic acid to *l*-dehydroascorbic acid. The *l*-dehydroascorbic acid is a dough conditioner and, in general, is said to be equivalent to bromate on a weight-for-weight basis. Burian (1939) has found that *l*-ascorbic acid retards the action of proteolytic enzymes, but unlike potassium bromate has no effect on the viscosity of the proteins and their degradation products, and thus does not influence the physical properties of the dough. Because of this latter fact, Burian states that the chemical treatment of flours with *l*-ascorbic acid should not be overrated.

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Although it appeared that *l*-ascorbic acid in grapefruit-juice bread would be poorly retained, experiments were performed investigating the fate of this nutrient and the effect of ascorbic acid from various sources upon the baking qualities of bread.

It has been reported that grapefruit juice is a valuable ingredient for retarding mold growth; however, this phase of the problem was not investigated.

Investigation of *l*-Ascorbic Acid Stability

The *l*-ascorbic acid was determined by the 2, 6-dichlorophenolindophenol titration method modified to use a solution containing 2% metaphosphoric acid and 6% acetic acid (Bessey, 1938). The dye solution was standardized against an *l*-ascorbic acid solution which in turn was standardized against a standard iodine solution.

Since there is some doubt as to whether the increase in *l*-ascorbic acid which may be produced by preliminary treatment with hydrogen sulfide is due to reduction of *l*-dehydroascorbic acid or the formation of other reducing materials, this procedure was omitted. The *l*-ascorbic acid values reported do not include any *l*-dehydroascorbic acid which may have been present. *l*-Dehydroascorbic acid has antiscorbutic properties similar to *l*-ascorbic acid (Borsook and co-workers, 1937). However, in slightly acid or neutral solutions, *l*-dehydroascorbic acid is readily converted to diketogluconic acid which is devoid of antiscorbutic properties. Any antiscorbutic value due to *l*-dehydroascorbic acid which might be contained in the baked products at the time consumed can only be definitely established by bioassay methods.

The *l*-ascorbic acid content of the grapefruit juice was determined by diluting 5.0 ml. to 50.0 ml. with a solution of the extracting acid and titrating without further treatment. Since the *l*-ascorbic acid value of the canned grapefruit juice determined by titrating in a mixture of 2% metaphosphoric acid and 6% acetic acid was within 3% of the *l*-ascorbic acid value determined by titrating in 8% acetic acid, it appears that the iron content of this canned grapefruit juice did not materially affect the *l*-ascorbic acid value determined by titrating in the presence of metaphosphoric acid.

The bakery products were prepared for titration in the following manner: A 5.0-g. sample was ground to a smooth paste with sea sand and 5.0 ml. of the solution of metaphosphoric acid and acetic acid. This mixture was made up to 30 ml. with the extracting acid in a graduated centrifuge tube, thoroughly mixed, then centrifuged. A suitable aliquot (usually 15 ml.) of the supernatant liquor was diluted to 25 ml. and titrated with the standard dye solution.

The following ingredients were used for both the straight and sponge dough processes:

	Percent on basis of total flour 100%
Flour	100
Water (approx.)	62
Salt	2
Yeast	2
Milk solids	4
Corn sugar	5
Shortening	4
Malt extract	1

Grapefruit juice in the quantities desired was used to replace the water in the formula. The amount of sugar in the formula was adjusted according to the amount of grapefruit juice used and the amount of sugar in the juice. In the sponge and dough process the grapefruit juice was added at the dough stage. When *l*-ascorbic acid was used, it was added to the formula given, dissolved in part of the water. In the sponge and dough method it was added at the dough stage. Semicommercial breadmaking technique was used throughout the experiments.

The formula for the cake used in the experiments is as follows:

	Percent on basis of flour 100%
Flour	100
Sugar	120
Shortening	30
Whole eggs	50
Grapefruit juice	95
Lemon flavor	9.75
Sodium bicarbonate	0.62
Salt	3
Baking powder	3.75

Discussion of Results

From the data in Table I it appears that conventional-type white bread (mix No. 1, control) contains approximately 0.003 mg. of *l*-ascorbic acid per gram. However, it is very doubtful if this should be considered as a true *l*-ascorbic acid value. It probably represents the difference between a blank for the 2, 6-dichlorophenolindolphenol titration in a solution of the extracting acid and the blank for the titration in the extracting acid plus constituents, extracted from bread, other than *l*-ascorbic acid.

The *l*-ascorbic acid contents of doughs containing added sources of *l*-ascorbic acid (mixes 2, 3, 4, and 5) from the mixer or bench were considerably less than the corresponding *l*-ascorbic acid values for the mix calculated from the weight of the mix and *l*-ascorbic acid added as grape-

fruit juice or crystalline *l*-ascorbic acid. The mixing of bread beats considerable quantities of air into the dough. In addition, the report of Melville and Shattock (1938) demonstrates an enzyme system in flour which is capable of promoting the oxidation of l-ascorbic acid to *l*-dehydroascorbic acid, which acts in a manner similar to bromates. Hence the lower *l*-ascorbic acid contents of the doughs which were found as compared with the *l*-ascorbic acid contents calculated from the formulas of the mixes indicate partial oxidation of *l*-ascorbic acid during mixing.

TABLE I

l-Ascorbic Acid Content of Breads

Mix No.	Type of bread	Weight of mix	Source of *l*-ascorbic acid	*l*-Ascorbic acid [1]	Sample from	*l*-Ascorbic acid found [2]
		lbs.		*mg./gm.*		*mg./gm.*
1	Straight dough	28.9	Control (none)	—	Mixer	0.003
1	Straight dough	28.9	Control (none)	—	Bench	0.004
1	Straight dough	28.9	Control (none)	—	Baked	0.003
2	Straight dough	27.3	2.25 lbs. grapefruit juice	0.030	Mixer	0.010
2	Straight dough	27.3	2.25 lbs. grapefruit juice	0.030	Bench	0.010
2	Straight dough	27.3	2.25 lbs. grapefruit juice	0.030	Baked	0.004
3	Straight dough	26.3	4.50 lbs. grapefruit juice	0.063	Mixer	0.033
3	Straight dough	26.3	4.50 lbs. grapefruit juice	0.063	Bench	0.032
3	Straight dough	26.3	4.50 lbs. grapefruit juice	0.063	Baked	0.006
4	Sponge dough	27.0	3.72 lbs. grapefruit juice	0.046	Second mix	0.034
4	Sponge dough	27.0	3.72 lbs. grapefruit juice	0.046	Baked	0.007
4	Sponge dough	27.0	3 72 lbs. grapefruit juice	0.046	24-hour bread	0.003
5	Sponge dough	27.0	600 mg. *l*-ascorbic acid	0.049	Second mix	0.030
5	Sponge dough	27.0	600 mg. *l*-ascorbic acid	0.049	Baked	0.003
5	Sponge dough	27.0	600 mg. *l*-ascorbic acid	0.049	24-hour bread	0.003

[1] Calculated from *l*-ascorbic acid content of grapefruit juice, or from weight of *l*-ascorbic acid added.
[2] Found by titration.

The *l*-ascorbic acid values of the order of 0.003 to 0.007 mg. per gram (ascorbic acid values in the blanks were almost equally as high), or 0.085 to 0.198 mg. per ounce, found for the baked breads with added grapefruit juice or *l*-ascorbic acid, compared with *l*-ascorbic acid values of the order of 0.030 to 0.063 mg. per gram, or 0.85 to 1.78 mg. per ounce calculated from the added grapefruit juice or *l*-ascorbic acid, indicate that the major portions of the added *l*-ascorbic acid were oxidized by the time the loaves were baked and cooled. The addition of grapefruit juice to bread does not appear to be practical as a means of increasing the *l*-ascorbic acid content of bread to a point where bread may be relied upon as a source of *l*-ascorbic acid of appreciable value in supplying the human requirement for vitamin C, which is of the order of 30 milligrams of *l*-ascorbic acid per day. The results are not in agreement with the finding of Zavyalov and Zavyalov (1938) that *l*-ascorbic acid in concentrated extracts of briar roses or of coniferous

needles added to bread is retained in the extent of 60% to 70% of the added *l*-ascorbic acid.

Cake batter and baked cake were more easily prepared for *l*-ascorbic acid titration than were bread dough and baked bread.

Data presented in Table II indicate that batter of commercial-type layer cake contains no appreciable amounts of *l*-ascorbic acid. The *l*-ascorbic acid content of batter with added grapefruit juice was found to be 0.077 mg. per gram as compared with 0.078 mg. per gram calculated from the weight of the batter and the *l*-ascorbic acid content of the added grapefruit juice. The baked cake was found to contain 0.039 mg. per gram. The *l*-ascorbic acid content of the cake decreased during storage, until 24 and 48 hours after baking the *l*-ascorbic acid contents of 0.023 and 0.021 mg. per gram, respectively, were found.

TABLE II

l-Ascorbic Acid Content of Cake

Mix No.	Type of cake	Total wt. of mix	Source of *l*-ascorbic acid	*l*-Ascorbic acid [1]	Sample	*l*-Ascorbic acid found [2]
		lbs.		*mg./g.*		*mg./g.*
1	Layer	—	None	0.000	Batter	0.000
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	Batter	0.077
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	Baked	0.039
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	24-baked	0.023
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	48-baked	0.021

[1] Calculated from *l*-ascorbic acid content of grapefruit juice.
[2] Found by titration.

The better retention of *l*-ascorbic acid in cake, as compared with bread, may be associated with less intensive mixing, the use of relatively smaller quantities of flour (hence smaller quantities of ascorbic acid oxidase), and a somewhat shorter baking time with cake as compared to bread.

The cake made from the formula containing grapefruit juice was of high quality and pleasing flavor.

The Dough-Conditioning Value of *l*-Ascorbic Acid, *d*-Ascorbic Acid, and Grapefruit Juice

The above work shows that the *l*-ascorbic acid content of baked bread prepared from formulas containing synthetic *l*-ascorbic acid or grapefruit juice was negligible. This indicates that the inclusion of *l*-ascorbic acid in bread formulas does not increase materially the antiscorbutic properties of the baked product. Therefore it was decided

to include *d*-ascorbic acid, which has no antiscorbutic value, as well as its optical isomer, *l*-ascorbic acid (vitamin C), in the baking quality tests.

In the experimental procedure both laboratory and commercial baking tests were performed. The laboratory test was designed to determine the true baking quality of the flour (including the effect of other ingredients) without the aid of enrichening ingredients. The commercial test was used to show how the flour, etc., would behave in a commercial bakery. Enrichening ingredients such as sugar, milk, and shortening were used in making the commercial loaves. Many tests were made; however, only typical examples are given in the results herewith presented.

Results and Discussion

Results are not given on grapefruit juice, for in quantities furnishing approximately 5 mg. of *l*-ascorbic acid per 100 g. of flour [3] (approximately 15% grapefruit juice on the basis of flour), the dough-conditioning value of *l*-ascorbic acid was apparently overshadowed by the effect of the citric acid content. This probably is due to a decrease in the gas-retaining properties of the gluten. Smaller quantities might have been of value in conditioning the dough.

Recommendations for making grapefruit-juice bread are to use approximately 15% on the basis of flour as 100%. However, if grapefruit juice is to be used to increase the nutritive properties of the bread, the largest quantities possible should be used. Thus quantities varying from 7.5% to 30% were tried, and in all these concentrations it decreased volume and gave a more dense texture in proportion to the quantity used. With the straight-dough method it was almost impossible to produce a loaf with properties similar to the control loaf, even with the lowest percentage. The sponge and dough method (grapefruit juice put in at dough stage) yielded much better results; however, it was necessary to give the dough a minimum amount of fermentation time. In view of these results grapefruit juice would probably not be desirable as a regular ingredient in white bread, but can be used for producing a special product.

Table III shows typical results of the effect of potassium bromate, *l*-ascorbic acid, and *d*-ascorbic acid on bread. All loaves were scored relatively for external and internal characteristics (100 points being best score for each) and volumes measured.

The flour used had a definite bromate response as is shown by the results for potassium bromate in Table III. The *l*-ascorbic acid was just as effective as bromate in improving the bread, while *d*-ascorbic had

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[3] Melville and Shattock (1938) showed that this quantity of *l*-ascorbic acid had a definite improving action on bread.

little effect. The *d*-ascorbic acid seemed to be detrimental except in quantities of 1.5 mg. per 100 g. flour. The same trend in results is shown by both the experimental and commercial loaves. The slight difference between the experimental and commercial loaves is, undoubtedly, due to a difference in formula—sugar, shortening, and milk being used in the commercial samples. Other experiments confirmed the results of Table III.

TABLE III

EFFECT OF BROMATE, *l*-ASCORBIC ACID, AND *d*-ASCORBIC ACID ON BREAD QUALITY
Quantities given in mg. per 100 g. flour. High grade
unbleached and unmatured flour used

	Stand-ard	KBrO₃				*l*-Ascorbic				*d*-Ascorbic			
	—	0.5	1.5	3.0	6.0	0.5	1.5	3.0	6.0	0.5	1.5	3.0	6.0
Experimental loaves:													
Volume in cubic inches	91.0	88.0	100.0	91.5	99.0	98.5	100.0	98.5	100.5	88.0	93.5	83.5	86.5
External score	94	98	99	93	97	100	100	100	99	91	96	91	89
Internal score	88	96	99	94	95	93	97	98	100	89	91	84	84
Commercial loaves:													
Volume in cubic inches	178.0	181.0	184.0	194.5	184.0	181.3	188.0	188.5	188.0	176.0	179.0	171.5	169.5
External score	92	94	100	98	98	93	96	97	97	85	90	88	87
Internal score	89	94	96	98	100	88	99	96	97	83	92	86	81

Experiments using a flour that was properly bleached and matured at the mill gave indications that potassium bromate was slightly different in its action from *l*-ascorbic acid. It was possible to add more *l*-ascorbic acid than potassium bromate without showing detrimental results; that is, the flours seemed to have greater tolerance to *l*-ascorbic acid than to potassium bromate. This might prove to be a slight advantage for *l*-ascorbic acid.

These results confirm the work of Jørgensen, Melville, and Shattock, and Burian. However, there was no indication that with the flours used (various grades) *l*-ascorbic acid was limited in its effect as compared to potassium bromate, as Burian pointed out.

General Conclusions

l-Ascorbic acid added to bread as crystalline ascorbic acid or as grapefruit juice appears to be rapidly oxidized during mixing and baking operations.

The *l*-ascorbic acid content of baked bread to which *l*-ascorbic acid had been added as crystalline *l*-ascorbic acid or as grapefruit juice was

found to be negligible and similar to that of conventional-type white bread.

Addition of grapefruit juice to bread does not appear to be justifiable from the viewpoint of increasing the *l*-ascorbic acid content of the baked bread.

Freshly baked cake retained approximately one-half, and cake 24 to 48 hours after baking retained slightly more than one-fourth, of the *l*-ascorbic acid in the added grapefruit juice.

Research of previous investigators which indicates that *l*-ascorbic acid is a dough conditioner equivalent to bromate, on a weight-for-weight basis, is confirmed.

d-Ascorbic acid is ineffective as a dough conditioner.

It is not possible to use grapefruit juice (in quantities tried, 7.5% to 30% on the basis of flour) as a source of *l*-ascorbic acid as a dough conditioner because of the detrimental effect of its acidity on the volume and texture of the bread.

Although grapefruit juice is not of value for increasing the *l*-ascorbic acid content of bread, it may have some value as a dough conditioner when used in small quantities.

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THE RELIABILITY OF AN AUTOMATIC KERNEL COUNTER FOR 1,000-KERNEL-WEIGHT DETERMINATIONS

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Investigations by the Agricultural Marketing Service of the malting quality of barleys have shown a need for an automatic device to count barley or malt kernels for 1,000-kernel-weight determinations. A simple automatic apparatus that can be used in this determination to give consistent and reasonably accurate results will be of great service to malting and brewing laboratories.

The Lefco Automatic Kernel Counter shown in Figure 1, designed
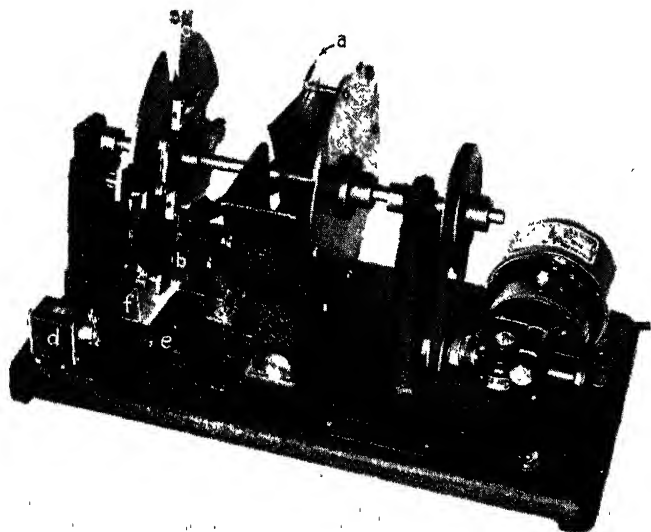
Fig. 1. Lefco Automatic Kernel Counter.—a, fingers for picking up one kernel at a time; b, stationary cam block with kernel notch and radial grooves; c, lever arm with wedge fins to move kernel out of notch and a cam knob to actuate counter arm; d, counter with automatic cut-off switch; e, box (in outline) for counted kernels; f, pan for discarded kernels.

for barley and malts and operating on mechanical principles only, has been tested in the course of our investigations. The operation of this device consists of picking up one kernel at a time from a hopper by radially moving fingers and then counting each kernel into a receptacle by means of cam and lever actions.

The fingers are designed to eliminate broken kernels and other seeds.

Each kernel acts as an actuating cam as it is moved along the face of the stationary cam block by means of a radially moving lever arm. The cam block has radially cut grooves and a special notch to place each kernel in a vertical position. The lever arm is held in place by spring action and carries wedge fins that pass through the grooves in the cam block to move each kernel on the face of the block. The lever arm also carries a knob on the back as a cam to activate the arm of the counting mechanism as the moving kernel forces the lever arm away from the stationary block. Should two kernels be in the notch at the same time, the top kernel will force the lever arm away from the face of the block far enough to discard the bottom kernel into a pan. The driving motor of the device is automatically cut off at the indicated count of 500 and 1,000 on the Veeder intermittent counter.

The accuracy of 1,000-kernel-weight values obtained by use of an automatic counter depends primarily upon the ability of the counter to be nonselective of kernel size in accordance with the work of Shands (1937) [2] on the standard method of analysis. The effect of inaccurate counting of kernels on the weight values will increase as the 1,000-kernel-weight values increase. The importance of the time required to make the automatic count depends upon the number of determinations to be made. Preliminary investigations with the counter showed that duplicate 1,000-kernel-weight determinations on the basis of two 500-kernel counts for each determination were in remarkably close agreement. This close agreement was maintained even when the variations between duplicate 500-kernel determinations were slightly greater than the one-gram allowable error of the official hand-counting method. This work also indicated that samples of approximately 60 g. for eastern barleys and approximately 75 g. for western barleys were sufficient for the determination.

A study of the reliability of the counter for making the determination has been made by comparison of results so obtained with those obtained by the official hand-counting method. Barley samples having a large range in 1,000-kernel weight and samples having a large range in kernel sizes within the samples were used.

Samples and Methods

For the purpose of this study 10 eastern barley samples consisting of 9 six-row and 1 two-row samples, and 10 western barley samples consisting of 8 six-row and 2 two-row samples were selected on the basis of a wide range in test weight per bushel. Dockage was removed from all the samples, after which they were mixed thoroughly

[2] H. L. Shands, Barley and malt studies: III—the determination of kernel weight, Cereal Chem. 14: 532–539, 1937.

and divided into two portions by means of the Boerner Divider. One set of the eastern-barley portions was given odd numbers from 1 to 19 and reserved for analysis. The corresponding portions were given even numbers from 2 to 20 and were analyzed for percentages of various kernel sizes by means of a new experimental drum sizer. The percentages of thin kernels passing through the drum sieve with slots 0.0761-inch wide were discarded, thus making samples 2 to 20 representative of malt-house barleys. Hereafter samples from which thin kernels have been removed will be designated "thinless." The data on kernel-size separations of the thinless samples are shown in Table I.

TABLE I

DISTRIBUTION OF KERNEL SIZES IN THE EASTERN BARLEYS BY WEIGHT PERCENTAGES

Sample No.	Total sound kernels	Thin kernels	Size determinations after removing thin kernels					
			Total sound over 0.0761 in.[1]	Through 0.0859 in.[1]	Through 0.0937 in.[1]	Through 0.1015 in.[1]	Through 0.1093 in.[1]	Over 0.1093 in.[1]
	%	%	%	%	%	%	%	%
2	96.7	3.9	98.0	14.6	22.9	34.4	21.2	6.9
4	97.0	2.2	97.6	11.9	18.4	35.5	25.6	8.6
6	92.3	19.7	94.2	37.1	28.0	23.0	10.3	1.6
8	95.0	7.0	96.4	19.0	23.2	31.7	19.0	7.1
10	95.5	8.8	96.6	23.4	26.0	30.9	15.6	3.9
12	· 90.8	58.0	96.4	63.0	18.5	12.2	5.0	1.3
14	88.4	12.9	95.0	25.7	25.6	28.4	15.8	4.5
16	96.3	8.3	97.6	22.7	25.9	31.1	16.0	4.3
18	91.3	2.0	94.1	8.6	16.1	34.7	29.0	11.6
20	93.7	2.8	93.8	4.9	5.9	17.5	36.7	35.0

[1] Sieve dimensions correspond to 4⅞, 5⅜, 6, 6½, and 7 sixty-fourths of an inch respectively, as used in barley graders.

The data show that the original samples had a range from 3.0% to 11.6% of material other than whole and sound barley kernels. This range was reduced to values from 2.0% to 6.2% by removal of the thin kernels. The samples are shown to have considerable variation in kernel size both between the samples and within each sample. Sample No. 12 is the only sample in which more than 50% of kernel weight occurred in one size classification. Obviously, the original samples containing thin kernels would have a much greater range in kernel sizes.

The western barley samples were not analyzed for kernel sizes. Odd numbers from 21 to 39 were assigned to one set of the portions, which were analyzed for 1,000-kernel weight by both methods. After making the tests the thin kernels were removed by means of the 0.0859-inch drum sieve, simulating malt-house procedure. The percentages

of thin kernels ranged from 3.2% to 44.1% in the original samples. The "thinless" samples were given the corresponding even numbers from 22 to 40. The two thinless samples later found to have the lowest and highest values for 1,000-kernel weight were sieved with the 0.1093-inch drum sieve to determine the percentages of extremely large kernels. The respective values were 19.0% and 62.1% for kernels failing to pass through this sieve.

The samples described above afford enough variation in 1,000-kernel weight, kernel size, and other factors to make a fair test of the reliability of the counter. In order to determine whether or not the counter action is affected by the size of the kernels, two series of 60-g. samples were prepared for analysis from the size separations of the eastern barleys. The first series consisted of eight samples having definite ratios by weight of different kernel sizes. Three samples contained two kernel-size limitations and five samples contained three size limitations. The 1,000-kernel-weight values were determined by making two 500-kernel counts with the counter and by hand counting whole barley kernels in the entire sample. The ratios of kernel sizes in the samples used and results of the two determinations are shown in Table II.

TABLE II

COMPARISON OF 1,000-KERNEL-WEIGHT DETERMINATIONS BY COUNTER AND BY
HAND COUNT OF TOTAL SAMPLE ON EASTERN BARLEYS WITH
DEFINITE WEIGHT RATIOS OF KERNEL SIZES

Sample No.	Weight ratio of kernel sizes in each sample as limited by sieve widths of —					1,000 kernel weight		Differences	
	0.0761 to 0.0859 in.[1]	0.0859 to 0.0937 in.[1]	0.0937 to 0.1015 in.[1]	0.1015 to 0.1093 in.[1]	Over 0.1093 in.[1]	Av. of duplicate counter tests	Hand count all barley in total sample	Between duplicate counter tests	Av. counter test minus hand count
						g.	g.	g.	g.
A	3	1	—	—	—	23.1	22.6	0.6	+0.5
B	1	1	—	—	—	25.6	25.4	0.3	+0.2
C	1	3	—	—	—	27.4	27.4	0.0	0.0
D	—	—	4	1	1	34.3	34.4	0.2	−0.1
E	—	—	3	2	1	34.4	34.5	0.4	−0.1
F	—	—	1	1	1	37.2	37.3	0.0	−0.1
G	—	—	1	4	1	38.0	38.0	0.3	0.0
H	—	—	1	2	3	40.6	41.2	0.8	−0.6

[1] Sieve dimensions correspond to 4¾, 5¼, 6, 6½ and 7 sixty-fourths of an inch respectively, as used in barley graders.

The variation in 1,000-kernel-weight values between duplicate 500-kernel tests as determined by the counter ranged from 0.0 g. to 0.8 g. The largest variations were for the samples having one of the kernel-size separations in the greatest proportion. The counter values

differed from the true values, obtained by hand counting the entire sample, by +0.5 g. for the lowest kernel-weight sample to −0.6 g. for the highest kernel-weight sample. The greatest differences occur with the samples having the largest proportion of kernels by weight and number within one-size separations of very small and very large kernels. For the entire range of 1,000-kernel-weight values the variations of the counter values from the hand values are well within the error allowed by the standard method.

The second series consisted of five samples having only kernels of one-size separation. Counter determinations were made as in the case of the first series of samples. The determination by the official method was used as the true value for making the comparisons. The whole barley kernels counted by the counter were checked and any other seeds present in the counted portion noted. The data on this series are shown in Table III.

TABLE III

COMPARISON OF 1,000-KERNEL-WEIGHT DETERMINATIONS BY COUNTER AND BY HAND ON EASTERN BARLEY SAMPLES HAVING ALL KERNELS LIMITED IN SIZE

Sample No.	Range in width of kernel [1]	No. kernels [2]	Weight		Actual composition of portion counted by mechanical counter			Differences		
								Between duplicate tests		Av. counter test minus av. hand test
			Counter	Hand	Barley	Oats	Other seeds, etc.	Counter	Hand	
	In.		*g.*	*g.*	*No.*	*No.*	*No.*	*g.*	*g.*	*g.*
I	0.0761–0.0859	500	10.7	10.7	514	4	1	—	—	—
		500	10.8	10.8	495	4	0	0.2	0.2	—
		1,000	21.5	21.5	1,009	8	1	—	—	0.0
J	0.0859–0.0937	500	14.6	14.5	501	2	0	—	—	—
		500	14.6	14.5	500	3	0	0.0	0.0	—
		1,000	29.2	29.0	1,001	5	0	—	—	+.2
K	0.0937–0.1015	500	16.9	17.1	500	0	0	—	—	—
		500	17.0	17.0	502	0	0	0.2	0.2	—
		1,000	33.9	34.1	1,002	0	0	—	—	−.2
L	0.1015–0.1093	500	19.4	19.2	498	0	0	—	—	—
		500	19.6	19.4	501	0	0	0.4	0.4	—
		1,000	39.0	38.6	999	0	0	—	—	+.4
M	Over 0.1093	500	24.6	24.7	500	0	0	—	—	—
		500	24.8	24.7	500	0	0	0.4	0.0	—
		1,000	49.4	49.4	1,000	0	0	—	—	0.0

[1] Sieve dimensions correspond to 4¾, 5¼, 6, 6¼, and 7 sixty-fourths of an inch respectively, as used in barley graders.
[2] In the case of the counter this refers to the registered number of kernels. In each case the 1,000-kernel portion is the two 500-kernel portions combined.

The range of 1,000-kernel-weight values is from 21.5 g. to 49.4 g., which is greater than the range for the first series of samples. These data indicate a high degree of accuracy for the kernel count. The counter failed to indicate nine barley kernels, eight oat kernels, and one other seed for sample No. 1. This, however, had very little effect on the weight value as indicated by the perfect check with the standard-method value. The variations in 1,000-kernel-weight values by the counter method from the values by the hand method are of the same order as those between duplicate tests by both methods and do not show a tendency to vary with the kernel-weight values. The comparisons indicate that for samples with all the kernels limited to a small range in size the counter can give reliable results for a large range in 1,000-kernel weight.

The 20 samples of eastern barleys previously described were analyzed by the counter method and the time required for the count on the 10 samples having no thin kernels was recorded in each instance. The number of kernels passing through the counter was checked for each sample. The entire test portions were returned to the samples before analyzing by the standard method. The time required for quartering and hand counting each of the 10 thinless samples was recorded. The comparative data on all the samples are shown in Table IV.

The 1,000-kernel-weight values ranged from 17.8 to 41.9 grams for these samples. The average variations between values for duplicate 500-count tests are 0.39 g. and 0.24 g. for the counter and standard methods, respectively. The differences between the 1,000-kernel-weight values by the two methods are not greater than 1 gram, the counter values ranging from 1.0 g. higher to 0.7 g. lower than the hand values. The extreme differences occur, as would be expected, with original samples containing thin kernels. The counter values average 0.26 g. greater than the hand values for the entire group of samples, but for the thinless samples the average difference is only −0.01 g. and the range is from +0.6 g. to −0.5 g.

The differences cannot be attributed to any inaccuracy of the kernel count because positive and negative differences occur in cases where the counter included more as well as when it included less than the recorded 1,000 kernels. The count on the thinless samples is very accurate considering that removal of thin kernels did not remove all broken kernels and other seeds from the samples. The agreement between the values for 1,000-kernel weight for the two methods is good for all the samples and particularly so for the thinless samples. These comparisons have been made without taking into account any

TABLE IV

TESTING THE KERNEL COUNTER WITH EASTERN BARLEYS

Sam-ple No.	Type of barley, 2- or 6-row	1,000 ker. wt., av. duplicate tests by		Differences in 1,000-kernel-weight values			Hand check on auto-matic count of 1,000 kernels	Counting time in minutes and seconds		
				Duplicate tests		Av. counter minus av. hand		Counter	Hand [1]	Diff., counter minus hand
		Counter	Hand	Counter	Hand					
		g.	g.	g.	g.	g.				
1 [2]	6	30.1	29.1	0.2	0.2	+1.0	1,005	—	—	—
2 [3]	6	30.7	30.5	0.2	0.2	+0.2	994	10:35	9:30	+1:05
3	6	33.1	33.0	0.6	0.0	+0.1	1,005	—	—	—
4	6	32.7	33.2	0.6	0.0	−0.5	1,007	14:20	11:57	+2:23
5	6	25.6	24.9	0.0	0.6	+0.7	975	—	—	—
6	6	29.0	28.8	0.0	0.4	+0.2	992	13:50	12:30	+1:20
7	6	29.0	28.7	0.8	0.6	+0.3	995	—	—	—
8	6	29.8	30.2	0.4	0.4	−0.4	997	13:00	11:10	+1:50
9	6	28.3	28.2	0.6	0.0	+0.1	1,004	—	—	—
10	6	30.3	29.7	0.2	0.2	+0.6	1,002	13:00	11:45	+1:15
11	6	18.1	17.8	0.2	0.4	+0.3	1,028	—	—	—
12	6	24.1	24.0	0.6	0.0	+0.1	991	14:15	11:10	+3:05
13	6	28.1	27.4	0.6	0.4	+0.7	947	—	—	—
14	6	29.4	29.5	0.4	0.2	−0.1	985	13:05	11:55	+1:10
15	6	28.4	27.4	0.4	0.0	+1.0	1,000	—	—	—
16	6	29.7	29.7	0.2	0.2	0.0	995	13:10	10:10	+3:00
17	6	33.7	32.9	0.6	0.6	+0.8	1,001	—	—	—
18	6	34.0	33.7	0.0	0.2	+0.3	1,000	13:10	10:45	+2:25
19	2	41.4	41.0	0.4	0.0	+0.4	985	—	—	—
20	2	41.4	41.9	0.8	0.2	−0.5	1,001	14:30	10:10	+4:20
			Av.	0.39	0.24	+0.26			Av.	+2:11

[1] Includes quartering time.
[2] Counter sample weighed from can.
[3] Both samples by quartering.

possible differences between the test portions due to sampling procedure.

The counter required an average of 2 minutes and 11 seconds more time to count two 500-kernel portions than was required by the hand method of quartering and counting the same number of kernels. The time comparisons were not made for continuous operations but on the basis of a single determination. It is impossible for an analyst to maintain the same speed for a large number of samples.

The samples of western barleys were used to furnish data similar to that determined on the eastern barleys with four exceptions in the procedure. The exceptions are: (1) The thinless samples were prepared from the portions tested as original samples. (2) For both methods the test portions of the thinless samples were selected by a Boerner Divider. (3) The total number of kernels was determined on the test portions used for counter tests on the original samples, and on the test portions used for both counter and hand tests on the

thinless samples. (4) The time data and number of whole kernels counted by the counter were recorded for the determinations on the 10 original samples. The data for the comparison of the counter method and official method are shown in Table V.

TABLE V

TESTING THE KERNEL COUNTER WITH WESTERN BARLEYS

Sample No.	Type of barley, 2- or 6-row	1,000 ker. wt., av. duplicate tests by		Differences in 1,000-kernel-weight values			Hand check on automatic count of 1,000 kernels	Counting time in minutes and seconds		
				Duplicate tests		Av. counter minus av. hand				Diff., counter minus hand
		Counter	Hand	Counter	Hand			Counter	Hand [1]	
		g.	g.	g.	g.	g.				
21 [2]	6	34.3	34.9	0.2	0.6	−0.6	994	21:57	15:00	+6:57
22 [3]	6	39.5	39.9	0.2	0.8	−0.4	—	—	—	—
23	6	38.7	39.5	0.9	1.0	−0.8	991	17:20	13:30	+3:50
24	6	43.0	44.4	0.0	0.7	−1.4	—	—	—	—
25	6	31.4	32.3	0.7	0.9	−0.9	986	17:30	13:00	+4:30
26	6	37.3	38.1	0.2	0.0	−0.8	—	—	—	—
27	6	49.2	49.7	1.1	1.0	−0.5	975	18:20	15:00	+3:20
28	6	49.4	50.9	1.0	0.0	−1.5	—	—	—	—
29	6	39.2	39.8	1.1	0.8	−0.6	994	16:28	11:40	+4:80
30	6	42.9	44.4	0.2	1.3	−1.5	—	—	—	—
31	6	47.1	46.9	0.9	1.0	+0.2	989	17:40	13:25	+4:15
32	6	47.4	47.9	0.3	0.7	−0.5	—	—	—	—
33	6	42.2	42.9	1.4	0.2	−0.7	978	14:58	12:35	+2:53
34	6	42.8	43.5	0.3	0.6	−0.7	—	—	—	—
35	6	40.5	41.1	0.8	1.0	−0.6	962	14:10	15:00	−0:50
36	6	41.8	42.4	0.1	0.3	−0.6	—	—	—	—
37	2	40.2	40.0	0.2	0.0	+0.2	985	13:10	14:30	−1:20
38	2	41.2	41.2	0.8	0.1	0.0	—	—	—	—
39	2	38.2	39.0	0.5	0.0	−0.8	992	13:25	14:00	−0:35
40	2	39.3	39.5	0.1	0.8	−0.2	—	—	—	—
			Av.	0.55	0.59	−0.66			Av.	+2:44

[1] Includes quartering time.
[2] Counter sample weighed from can.
[3] Both samples of minimum size from Boerner Divider.

The 1,000-kernel-weight values by the hand method show a range from 32.3 g. to 50.9 g. Removal of thin kernels increased the weight value as much as 5.8 g. for samples No. 25 and No. 26 where 44.1% of the original sample was thin kernels. The differences between duplicate tests are on the average 0.55 g. and 0.59 g. for the counter and hand methods, respectively. Differences equal to or greater than the allowable error of one gram occur in four instances for the counter method and in five instances for the hand method. Apparently close checks between duplicates are more difficult to obtain with this class of barleys.

The counter values average 0.66 g. lower than the hand values. Only two original samples show the counter values to be higher than

hand values. In the case of Nos. 24, 28, and 30, all thinless samples, the counter values are approximately 1.5 g. lower than the hand values; but two of these samples show a greater difference between duplicate hand tests than that shown for duplicate counter tests. However, all three of these samples were long-awned varieties. The check on the number of kernels in the automatic count on the original samples shows that only in three instances could the error in count be considered as the only factor causing the low values by the counter method. The time required by the counter averaged 2 minutes and 44 seconds longer than the hand count, on the basis of a single determination.

The weight of actual number of kernels determined as described in the procedure for these kernel-weight values was used as a basis to determine the average differences for each method and for each test sample. The data are shown in Table VI.

TABLE VI

COMPARISON OF THE 1,000-KERNEL-WEIGHT VALUES OF TABLE V WITH VALUES
DETERMINED ON THE BASIS OF THE TOTAL NUMBER OF KERNELS
IN THE SAMPLE

Sample No.	Automatic counter sample			Hand counted sample			Diff., total counter minus total hand
	1,000 ker. wt.		Diff., duplicates minus total sample	1,000 ker. wt.		Diff., duplicates minus total sample	
	Av. duplicate tests	Total kernels hand counted		Av. duplicate tests	Total kernels hand counted		
	g.	g.	g.	g.	g.	g.	g.
21	34.3	34.2	+0.1	34.9	—	—	−0.7
22	39.5	39.9	−0.4	39.9	39.9	0.0	0.0
23	38.7	39.3	−0.6	39.5	—	—	−0.2
24	43.0	43.4	−0.4	44.4	44.1	+0.3	−0.7
25	31.4	31.4	0.0	32.3	—	—	−0.9
26	37.3	37.4	−0.1	38.1	37.5	+0.6	−0.1
27	49.2	50.3	−1.1	49.7	—	—	+0.6
28	49.4	50.5	−1.1	50.9	50.4	+0.5	+0.1
29	39.2	39.6	−0.4	39.8	—	—	−0.2
30	42.9	44.1	−1.2	44.4	44.6	−0.2	−0.5
31	47.1	47.6	−0.5	46.9	—	—	+0.7
32	47.4	48.1	−0.7	47.9	47.6	+0.3	+0.5
33	42.2	42.9	−0.7	42.9	—	—	0.0
34	42.8	43.7	−0.9	43.5	43.5	0.0	+0.2
35	40.5	40.9	−0.4	41.1	—	—	−0.2
36	41.8	42.2	−0.4	42.4	42.0	+0.4	+0.2
37	40.2	40.3	−0.1	40.0	—	—	+0.3
38	41.2	41.3	−0.1	41.2	41.0	+0.2	+0.3
39	38.2	38.1	+0.1	39.0	—	—	−0.9
40	39.3	39.5	−0.2	39.5	39.5	0.0	0.0
		Av.[1]	−0.55		Av.[1]	+0.21	Av.[1] 0.0
		Av.[2]	−0.46				Av.[2] −0.075

[1] Only even numbered samples.
[2] All samples.

The total number of kernels in the hand-test samples ranged from 1,006 to 1,285 and in the counter test samples from 1,271 to 1,573.

The 1,000-kernel-weight values by the counter averaged 0.46 g. lower than the true values (based on the total number of kernels), for all of the samples. The average for the thinless samples is 0.55 g. lower. These average differences are influenced to a considerable extent by the large differences for long-awned samples, 27, 28, and 30. The range for the difference values for all samples is from +0.1 g. to −1.2 g., all but two of which are negative values.

The 1,000-kernel-weight values by the hand method on the thinless samples were 0.21 g. on the average higher than the true values. The difference values show the hand method to have a value lower than the true value in only one instance. The range in difference values was from +0.6 g. to −0.2 g. Since the comparison of the hand-method values to true values has been made on thinless samples only it is quite probable the average difference would have been even higher if the original samples had been included.

A comparison of the true 1,000-kernel-weight values for the counter samples and the hand samples shows that the true values of the counter samples averaged 0.075 g. lower than those of the hand samples, and that the range was from 0.9 g. lower to 0.7 g. higher.

Discussion of Results

The results from the samples in Tables II and III indicate that counter determinations are reliable for samples having considerable range in kernel sizes and in 1,000-kernel weight. The range of +0.5 g. to −0.6 g. deviation found with extreme conditions is in accordance with the deviation values shown by Shands (footnote 2). The comparative data in Table IV for samples of eastern barleys that represent dockage-free samples and malt-house samples show that counter determinations on the average may be expected to be 0.26 g. too high. This average and the range of variations shown indicate that the counter values can be relied upon to have approximately the same significance as the standard-method values. This is particularly true of the values for the malt-house samples.

The data on the western barley samples for the two methods show that the counter values on the average are 0.66 g. too low. The consistency of low values is significant.

However, the larger average of 0.66 g. and smaller range in the variations than shown by the data for the eastern barley samples do not indicate a material difference in the degree of accuracy for the counter determinations. The low values can perhaps be attributed to the increasing deviation in single-kernel weight for increasing size of

kernel, and the fact that an oat or similar material in these samples that was counted as barley, weighs considerably less than a barley kernel.

The data on the same samples in Table VI are based on the actual weight of the kernels in the two determinations and confirm the underestimation of 1,000-kernel weight by the counter method. The average error is reduced, however, from 0.66 g. to 0.46 g. On the other hand, the data show that the standard hand method has overestimated the 1,000-kernel weight on the average by 0.21 g. Two analysts making these determinations independently had a tendency to select larger than average kernels. This may be the case with most analysts with this class of barley.

The average difference between the samples based on the weight of all the kernels is 0.075 g. Therefore, taking into account either one or both of the values of 0.21-g. average overestimation by the hand method and the 0.075-g. average difference between the samples, we have a reduction of the 0.66-g. average underestimation by the counter determinations as shown by the comparison of methods in Table V.

It is quite probable that determinations on the basis of total number of kernels for the eastern barley samples would have shown low values for the official method and considerable difference between the samples tested by both methods. This assumption can be based on the fact that the eastern samples have a large proportion of small-size kernels and that the test portions were not mechanically selected.

Conclusions

The investigations on the reliability of the Lefco Automatic Counter for the determination of 1,000-kernel weight of barleys herein reported support the following conclusions:

1. Counter determinations on samples having all kernels within one small range of kernel size are equally reliable over a large range in 1,000-kernel weight.

2. A mixture of kernel sizes within a sample affect the counter determinations only when there is a large proportion of an extreme kernel size in the sample.

3. Counter determinations on dockage-free eastern barleys are consistently higher than official-method determinations over a large range of kernel-weight values.

4. Counter determinations on samples of eastern barley without thin kernels consistently check the official method determinations within the standard deviation of the official method.

5. Counter determinations on dockage-free samples and samples

without thin kernels of Western barleys are consistently lower than official-method determinations.

6. The variation of counter determinations from official-method determinations mentioned in conclusions 2, 3, and 5 are within the standard deviations of the official method.

7. The comparison of 1,000-kernel-weight values on the basis of all the kernels in the test portions for the western barley samples indicates that if smaller test portions had been used for the counter determinations on the eastern barleys and if these portions had been mechanically selected, even more accurate results would have been obtained.

8. The use of the automatic counter for 1,000-kernel-weight determinations removes any personal error and can maintain an efficiency for a number of determinations that it is impossible to attain by the official method.

TESTING THREE FLOURS IN FIVE CAKE FORMULAS

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The purpose of the cake-baking test is to differentiate flours. Ability to distinguish among flours should, of course, be reproducible in different laboratories. Three flours were selected for this work, mainly because of their differences in characteristics. Pup loaves of bread were made, and the tentative A. A. C. C. cake formula and four other cake formulas were used. The objects of this work were: (1) to correlate cake quality with flour analyses, and (2) to determine the type of cake formula which would measure differences among flours most satisfactorily.

Flour Characteristics

The three flours selected are referred to as X, Y, and Z. The analyses of these flours are shown in Table I.

X and Y were commercial cake flours widely known in the baking industry as excellent cake flours. The main difference between the two is the lower viscosity of Y. Flour Z was purposely selected because of its analysis. The high ash, protein, and pH, its viscosity characteristics, and coarse granulation indicate a poor flour for cake.

Figure 1 shows test loaves of bread baked from the three flours each

TABLE I

FLOUR ANALYSES

Flour	H_2O	Ash (15% m.b.)	Protein (15% m.b.)	pH	Viscosity, cc. lactic acid					20 min. granulation				(30 min.) Thru 250
					0	1	3	5	7	On 165	On 230	On 250	Thru 250	
X	11.65	0.339	7.68	5.13	4	30	38	42	42	—	1.0	41.0	58.0	83.0
Y	10.65	0.358	7.74	4.90	5	12	17	19	19	—	—	46.0	54 0	80.0
Z	12.20	0.501	8.89	5.52	15	13	36	45	46	—	6.0	54.0	40.0	62.0
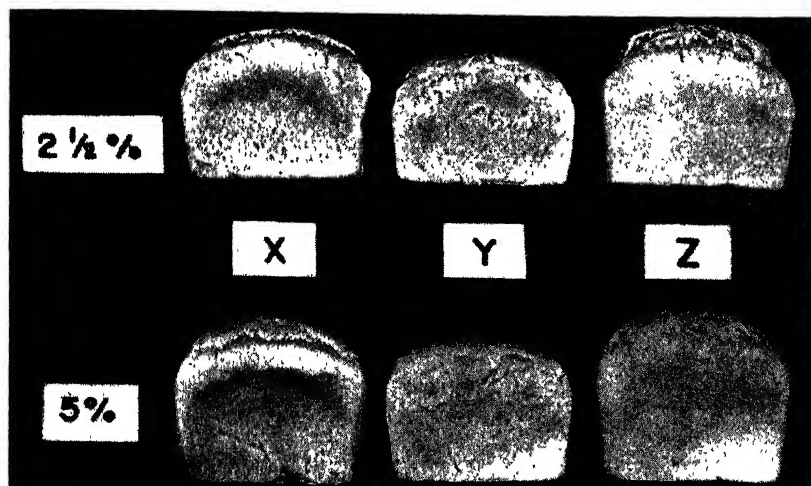
Fig. 1.   Loaves baked with 2½% and 5% of sugar.

at two sugar concentrations, $2\frac{1}{2}\%$ in the upper row and 5% in the lower row.   As might be expected, flour Z gave the largest loaf.

Cake Baking Tests

The five cake formulas used with the three flours are shown in Table II.

TABLE II

FIVE CAKE FORMULAS USED

Formula	Description	Flour	Sugar	Short-ening	Salt	Baking powder	Eggs	Milk
OT	Present tentative	100	96	25	1.5	4.0	32	88
OM	Modified tentative	100	96	35	1.5	4.0	35	88
P	Rich pound cake	100	120	70	3.8	0.0	70	50
YL	Rich yellow layer	100	140	55	3.8	6.3	60	105
WL	Rich white layer	100	140	55	3.8	6.3	75	95

Formula *OT* is the present tentative test method.   *OM* is a modified formula with slightly increased shortening and eggs and was one of the formulas used by the 1939–40 committee.   *P* is a rich commercial yellow pound cake, high in sugar, shortening, and eggs.   *YL* is a rich commercial yellow layer cake.   *WL* is a rich white layer formula almost identical with a formula used by the 1938–39 committee.   Because of the nature of the formulas, special cake shortening was used in each of the last three.

Figure 2 shows cakes made according to the present tentative test method.   The volumes of the cakes were almost identical, and the symmetry was good in every case.   The second cake had the best grain,
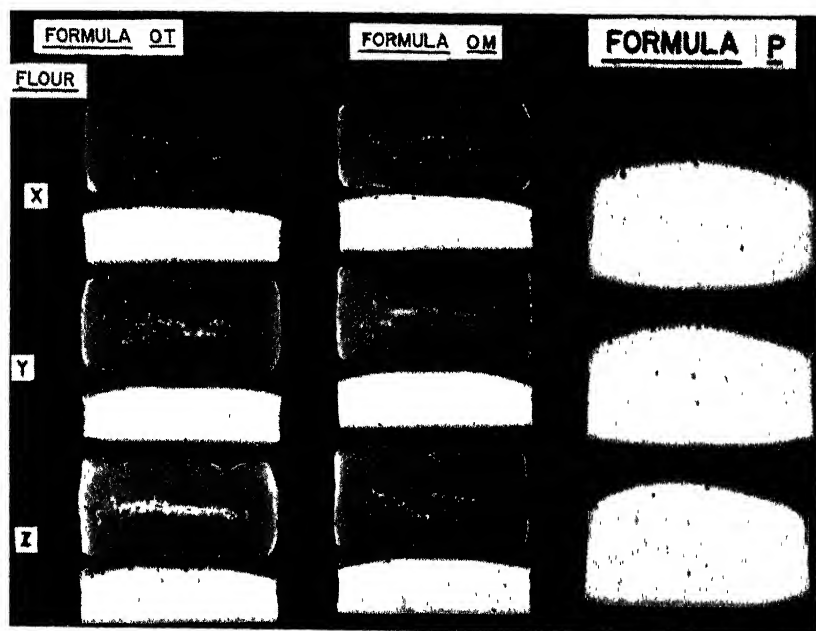
Fig. 2.   Present tentative method.

Fig. 3.   Modified formula with shortening and eggs slightly increased in comparison with formula *OT*.

Fig. 4.   Rich yellow pound cake formula.

but all three would be judged satisfactory.   Using the standard score sheet, *Y* showed the best score with 86, with *X* and *Z* practically equal at 81 and 80.

In Figure 3, showing formula *OM* which is not very different from the previous formula, the results are practically the same as in the case of formula *OT*.   *Y* is best with *X* and *Z* tied for second and third. The actual scores were 81 for *X*, 88 for *Y*, 82 for *Z*.

Figure 4 shows the three flours used in the rich yellow pound cake formula. With this formula, all three flours gave almost the same cake. That is, this cake formula did not distinguish any differences among the three flours. The actual scores of the cakes were 91, 90, and 92.

Figure 5 shows rich white layers made from the three flours. In this formula, flours X and Y gave cakes better in volume, grain, silkiness and color than flour Z. The scores of the cakes were 88, 90, and 83—in close agreement with what are generally considered as required analytical characteristics.
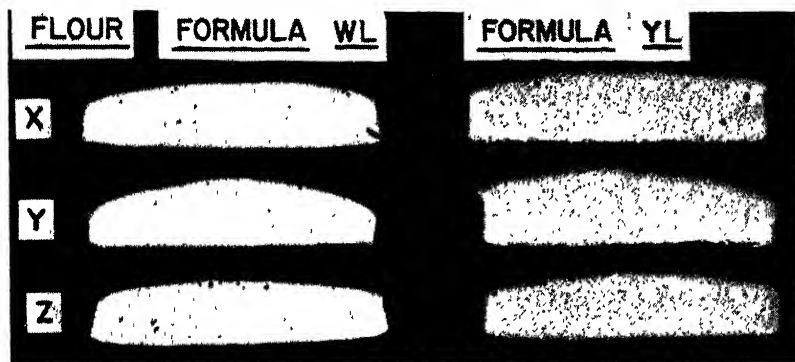
Fig. 5.   Rich white layer cake formula.   Fig. 6.   Rich yellow layer cake formula.

Figure 6 shows the rich yellow layer cakes. The volume of the cake made from flour Z is distinctly poorer than the other two but in other characteristics the cakes are practically equal.

Scoring

The scores of these cakes cannot be compared *between* formulas. Even though all of the scores of the 15 cakes fall in a fairly close range, there is a wide difference in their quality as judged from the bakers' and consumers' points of view. This is especially true if the formulas are compared from the standpoint of eating quality. If only one cake formula is considered, eating qualities are important only when the flour produces differences in eating quality. On the other hand, when various cake formulas are considered, as in this work, the official scoring system is not flexible enough to show these large differences in eating quality. Standards generally accepted for scores on the official cake also do not allow these differences to be shown.

Conclusion

Based on this study, it is indicated that:

1. Flours which are decidedly inferior in one cake formula may be entirely satisfactory in another.   Relative ranks by one formula may be markedly changed if another cake formula is used.

2. Analytical data ordinarily obtained are not sufficient to predict the suitability of a cake flour for a variety of cake formulas.

THE EFFECT OF SHORTENING ON THE A. A. C. C. BREAD–BAKING TEST

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Shortening has not been included among the ingredients used in the A. A. C. C. baking test formula for bread, although it is a common constituent of commercial bake-shop formulas.   A perusal of the literature pertaining to experimental baking reveals that, even though lacking official sanction, a great many of the baking results reported in connection with various phases of cereal chemistry have been made with formulas which included shortening.   Indeed it is surprising that the use of shortening in the experimental baking test has not become even more prevalent than it is since various treatises and books on baking exalt its usefulness and claim such virtues as improved loaf volume, better appearance, softer crumb, and more silky texture when shortening is incorporated in the dough.

One of the obvious reasons for omitting fats from the experimental baking test has been the desire to avoid the introduction of another variable.   It is generally recognized that there is considerable difference in the shortening power of various types of fat.   Heald (1937) reported the differences in loaf volume resulting from the use of equivalent quantities of animal, vegetable, and compounded shortening in a bread formula.   However, there appears to be no information available which precisely indicates the variation in loaf volume and bread characteristics

which can be expected to result when various types of commercial short-ening are included in the basic A. A. C. C. baking formula.

Review of Previous Work

Shortening as an ingredient of the basic A. A. C. C. baking test formula has attracted some attention. Merritt, Blish, and Sandstedt (1932) reported that shortening added to the formula increased loaf volume. As indicated previously, Heald (1937) reported the effect of different animal, vegetable, and compounded fats on loaf volume, grain, and texture when the quantity of shortening in each case varied over a range from zero to 12%. It was found that the maximum reaction was obtained when the dough contained 4% shortening and all classes of fat caused an increase in loaf volume and better internal loaf characteristics.

The effect of shortening on the gas production and retention of doughs has also been studied by Heald (1939). He found that the rate of gas production was retarded by the inclusion of shortening in the formula. Gas retention, however, was found to be influenced by the flour grade and bleach as well as by the inclusion of shortening; therefore, no general rule can be formulated which will hold for all cases.

Heald (1939) recommends that shortening be included in the A. A. C. C. baking formula; and Sandstedt and Blish (1939), following their study of the influence of sugar levels on the baking value of commercial flours, make a similar recommendation.

From the review of past accomplishments it appeared that additional information should be obtained before the inclusion of shortening in the basic baking-test formula could be safely recommended. Information concerning the influence of different types of fat must of necessity be available, since obviously, if the shortenings regularly purchasable on the market produce a significant difference in baking results, then the problem of recommending shortening for the baking formula becomes complicated by the additional task of designating a specific type of fat.

It is unfortunate that more of the recent researches in physical chemistry are not immediately applicable to the study of dough. Platt and Fleming (1923), however, have discussed some of the surface phenomena involved in the use of shortening in a dough. The idea of molecular orientation developed by Hardy (1913) and demonstrated by Langmuir (1917) has thrown important light on many of the fundamental problems related to shortenings and lubricants. Because of the complexity and diversity of the surfaces in a bread-dough system and the known variation in the surface area covered per unit quantity of fat, depending upon such variables as the degree of unsaturation, number and kind of polar groups, and the properties of the surface, it is

difficult to present a truly adequate picture of the distribution of fat in a dough. For practical purposes the action of fat in a dough during mixing can be visualized as the internal lubricant. The fat spreads on the gluten and starch surfaces and coats them with a thin film of grease, thus separating the dough constituents and permitting greater ease of slippage between the different parts.

Experimental

The effects of shortening on dough development.—A micro recording mixer was used to study the effect of shortening on dough development. Salt, sugar, and yeast doughs, with and without the addition of shortening, were compared. Figure 1 shows typical curves. The upper curve indicates the mixing characteristics when no fat was included in the dough, and the lower curve shows the characteristics of a similar dough but including 3% of a hydrogenated shortening introduced into the mixing bowl at 80°F.

It is evident that the inclusion of fat in a dough lowers its consistency but does not materially alter the rate of development. There is a tendency for the break-down of the gel structure to be less drastic when fat is present as is illustrated in Figure 1. The two curves almost coincide
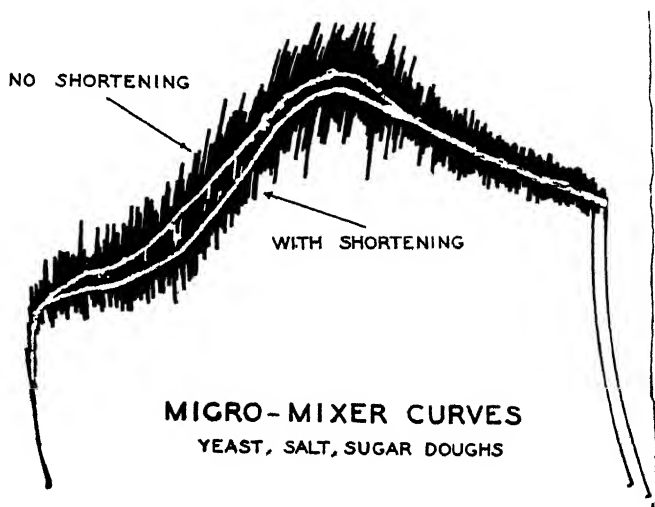
Fig. 1. The effect of 3% of shortening on dough properties as indicated by a micro recording mixer.

after a similar mixing period, although at the peak development point the nonfat curve has a higher consistency and therefore the steeper slope on the falling-off portion of the curve.

It seems apparent from a study of recording dough-mixer curves that the inclusion of shortening in the baking test would involve no readjustment insofar as mixing considerations are concerned. The validity of this statement is strengthened by the fact the National Micro Recording Dough Mixer has the same type of dough-developing action as the Swanson-Working (1936) mixer.

The effect of different shortenings on loaf volume and bread score. —Seven shortenings were obtained from common commercial sources. The samples represented typical vegetable, animal, and compounded fats, a price differential between samples within a given type, and one liquid shortening. Effort was made to have sufficient diversity of selection to include any type such as might be purchased for the average cereal laboratory. Following the recommendation of Heald (1939) 3% of each of these shortenings was included in the baking formula and baking tests conducted according to the method outlined in Cereal Laboratory Methods (1935).

Two different flour grades were used for the baking test. One was a baker's patent with the following analysis on a 15% moisture basis: protein 12.8%, ash 0.40%, absorption 63.0%, diastatic activity 310 mg. maltose. The other was a straight-grade family flour corresponding to the following description: protein 10.6%, ash 0.42%, absorption 60.3%, diastatic activity 234 mg. maltose. Each time the experiment was repeated duplicate doughs were baked using each shortening, and the entire series of fats was always included in one bake. The relative position of the doughs containing the different shortenings were progressively changed in respect to one another in the baking sequence in order to avoid errors which might occur if the doughs maintained fixed positions in the series. The entire shortening series was rebaked 20 times with the baker's flour and 10 times with the family flour.

Results

The results of this investigation are recorded in Tables I and II. Loaf volume and bread score were recorded as criteria of the change produced by different fats. The bread score was obtained by assigning numerical values to the important external and internal loaf characteristics, including the volume. The mean loaf-volume and bread-score values reported for the seven shortenings and the control is the average of 40 loaves in Table I and 20 loaves in Table II. It is evident that the inclusion of shortening in the baking formula produces bread of greater volume and higher numerical score. In these experiments there was no indication that shortening tended to reduce the variability of either the

TABLE I

BAKER'S GRADE FLOUR

Significance of Differences between the Mean Loaf Volumes and Bread Scores for Shortening Types and those Obtained by an Individual Shortening

Shortening type	Loaf Volume (cc.)				
	Mean volume	Stand. deviation	Coef. of variation	Stand. error of mean	$\dfrac{D*}{E_D}$
None	731.5	24.11	3.30	5.392	3.896
A	756.6	21.53	2.84	4.813	0.178
B	761.2	21.67	2.85	4.847	0.448
C	761.6	24.27	3.19	5.428	0.574
D	767.5	27.15	3.54	6.071	1.349
E	756.5	26.43	3.49	5.909	0.163
F	759.4	20.69	2.72	4.626	0.290
G	767.2	27.38	3.57	6.124	1.295

Shortening type	Bread Score				
	Mean score	Stand. deviation	Coef. of variation	Stand. error of mean	$\dfrac{D*}{E_D}$
None	94.0	1.36	1.44	0.299	4.713
A	96.1	1.19	1.25	0.268	0.352
B	95.9	1.02	1.07	0.229	0.166
C	96.3	1.67	1.74	0.374	0.864
D	96.3	1.09	1.13	0.244	0.945
E	96.5	0.89	0.92	0.198	1.313
F	96.2	1.02	1.06	0.227	0.919
G	96.4	1.09	1.14	0.245	1.157

* D = Difference between means. $P = 0.05$
E_D = Standard error of difference between means. $N = 26$
Significant difference when $D/E_D > t$. $t = 2.056$

loaf volume or the bread score. In fact, the family-flour loaves baked without the addition of fat had the smallest coefficient of variation of loaf volume of the entire group.

Statistical analysis reveals a significant difference between the volumes of loaves obtained when shortening was included in the formula, compared to similar loaves baked from doughs without shortening. However, the different shortening types did not produce a significant difference. The bread scores followed this same rule and both flour types led to precisely the same result. In determining the probability that the difference between means is significant, use was made of Fisher's (1934) table of t values. A significant difference is one that gives a greater calculated value of t (see note at bottom of Tables I and II) than the corresponding reading from the table for $P = 0.05$.

Discussion

This investigation indicates that shortening could be included in the A. A. C. C. bread-baking formula without necessarily designating a specific type of fat. This statement should not be construed to mean

TABLE II

FAMILY GRADE FLOUR

Significance of Differences between the Mean Loaf Volumes and Bread Scores for Shortening Types and those Obtained by an Individual Shortening

Shorten-ing type	Loaf Volume (cc.)				
	Mean volume	Stand. deviation	Coef. of variation	Stand. error of mean	$\dfrac{D*}{E_D}$
None	630.8	12.57	1.99	3.975	4.919
A	657.7	33.17	5.04	10.490	0.235
B	661.7	23.28	3.52	7.361	0.156
C	669.2	26.40	3.94	8.349	1.047
D	664.3	15.94	2.40	5.040	0.579
E	661.1	20.57	3.11	6.504	0.009
F	668.0	18.32	2.74	5.793	1.037
G	670.3	19.32	2.88	6.111	1.266

Shorten-ing type	Bread Score				
	Mean score	Stand. deviation	Coef. of variation	Stand. error of mean	$\dfrac{D*}{E_D}$
None	89.3	1.32	1.47	0.465	2.887
A	91.6	1.51	1.65	0.522	0.776
B	91.1	0.98	1.07	0.830	0.107
C	91.7	1.67	1.83	0.579	0.935
D	91.4	1.58	1.73	0.547	0.457
E	91.8	0.98	1.06	0.335	1.174
F	91.2	1.00	1.10	0.348	0.218
G	90.7	0.79	0.87	0.275	0.578

* D = Difference between means. $\qquad$ $P = 0.05$
E_D = Standard error of difference between means. $\qquad$ $n = 16$
Significant difference when $D/E_D > t.$ $\qquad$ $t = 2.120$

that shortening can be used indiscriminately in the bread-baking test, regardless of composition, workability, consistency, or cost. It is essential to remember that the fats used in this experiment were incorporated in the doughs only at the relatively low level of 3% and no consideration was given to such important aspects as the keeping properties of these fats or their influence on taste, odor, or palatability of the bread. However, this experiment does indicate that at the 3% level there was no significant difference between the baking-test results obtained from the use of various types of high-grade shortening such as are readily obtainable on the market.

The literature is replete with references to the variability of animal and vegetable fats. In the interest of keeping conditions as constant as possible, it would be advisable to use an all-hydrogenated shortening, since these can be manufactured under rigid specifications, are widely distributed, and consequently available to practically every cereal chemist.

Summary

Shortening is extensively employed in test-bake formulas although it does not have the official sanction of the A. A. C. C. The suggestion that shortening be included among the ingredients in the basic bread-baking formula is not new, and the literature already contains references to the effect of fats on bread characteristics and on the gas production and gas retention of doughs. Also, information is available on the effect of different types of fats used at various levels on the properties of bread.

The present study represents an effort to supply additional information regarding the effects of the inclusion of fats on the A. A. C. C. basic bread-baking test. The influence of fats on the mechanical development of doughs had not been recorded and essential knowledge regarding the significance of differences in bread quality caused by the use of various types of commercial shortening seemed inadequate.

Shortening does not materially affect the rate of dough development as measured by the National Recording Micro Mixer; it does, however, reduce the detrimental effect of overmixing and decreases dough consistency.

Statistical analysis of the data obtained by rebaking a series of doughs varying only by the inclusion of 3% of seven different fats revealed that no significant variations occurred in either the loaf volumes or bread scores. A significant difference in bread characteristics was produced between the bakes containing shortening and those without fats. The inclusion of shortening in the baking-test formula will reduce the stickiness of doughs and produce bread of larger volume, better grain, and better texture.

It appears that shortening to the extent of approximately 3% can be safely recommended for inclusion in the A. A. C. C. basic baking-test formula without introducing a significant variable, even though no specified fat is mentioned. In the interest of uniformity, however, it would possibly be advisable to recommend an all-hydrogenated shortening.

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A MECHANICAL MEANS OF DETERMINING THE VALUE OF "VOLUME" ON A BREAD SCORE REPORT

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Many ways are used for reporting the volume of bread. In scoring commercial bread some operators simply look at the loaf and give it a value, which is included in a numerical score. Others merely report cubical content (in cubic inches or cubic centimeters) exclusive of the numerical score, while still others have devised methods of including the number of cubic centimeters of loaf volume in a total numerical score. Saunders, Nichols, and Cowan (1922), Blish (1928, 1929), and Larmour (1929) have discussed the two latter methods.

The following method has proved to be a simple one for quite accurately employing a "yardstick" (determined mechanically) for volume on a bread score report.

Commercial Bread Scoring

In scoring commercial bread, volume is not given as much consideration as in experimental scoring. The numerical score which has been in use at the Institute since 1922 has been rather widely accepted. This scorecard was developed by considering other scorecards and by a questionnaire to all members of the American Bakers' Association for their ideas (see Baking Technology **1**: 14, 42 (1922). In this scorecard loaf volume is assigned a maximum of 10 points out of 100.

It is evident that a value for volume based on appearance would lead to great error. It is also evident that an attempt to arrive at a value for volume for various loaves on the basis of their total cubical volume would be erroneous, due to the great variations of total weight with total volume. However, the value for volume can be based quite accurately on the volume of a unit weight of the bread. This will be referred to hereafter as unit volume. This might be either cubic inches per ounce or cubic centimeters per gram. There are several reasons for preferring the former:

1. *The average unit volume (cu. in. per oz.) of many loaves scored comes fairly close to 10.* The average unit volume of the loaves scored in the last 18 months at the Institute is 9.5 cu. in. per oz. of bread. Pirrie (1936) reported unit volume on loaves from various parts of the country with an average of 10.2 cu. in. per oz. of bread.

2. *The correct amount of dough for a certain pan will yield a loaf whose volume is approximately 10 cu. in. per oz.* It is well known that for a certain type of bread too large or too small a pan will give a resulting loaf of relatively poorer quality than the more nearly correct size. What is the optimum size?

Ingles (1937) reported some collaborative work of the Research Committee on the A. S. B. E. on the effect of pan sizes on the palatability (eating qualities) of bread. Palatability was considered to consist chiefly of odor, taste, and mouth feel. The results of this report confirm earlier findings by Pickering and Norton (1933) that 5.83 cu. in. of pan size per ounce of dough (round-top bread) are the most desirable, or produce the most palatable loaf. It was also concluded that: "These experiments seem to show that for 30 hour old bread, the pan dimension, whether longer or shorter, does not matter so much, just so long as we keep the pan size at 5.83 cubic inches per ounce of dough—but that dropping this to even 5.50 noticeably affected palatability."

Cathcart (1937) tested the effect of various factors of "make-up" on bread flavor. Flavor here was considered as the aroma and the taste of the bread. Pan sizes varying from 4.43 cu. in. per oz. of dough to 7.19 cu. in. per oz. of dough were tested. The bread was one-pound round-top loaves. Although the differences found in the flavor of bread baked in the various sized pans was slight, the results of the experiments favored the pan having a capacity of 5.97 cu. in. per oz. of dough as being the most desirable.

An average of the above factors (optimum cu. in. of pan volume per oz. of dough) is about 5.9. Table I shows typical results with round-top

bread using this factor for various sizes of pans. It will be noted that unit volumes of all the hand-made samples are 10 or over, while the machine-made samples are 10 or very close to 10.

TABLE I

Unit Volume of Round-Top Bread Resulting from Various Sizes of Pans
Factor 5.9 used to calculate scaling weight of dough. Medium rich formula
and good grade baker's patent flour used.

Exp. No.	Pan measurements in inches			Bread volume, cu. in. per oz.	Remarks
	Top inside	Bottom outside	Depth		
1	4½× 9	3¾× 8¼	3	10.5	Hand make-up
7	4½× 9	3¾× 8¼	3	10.5	Hand make-up
17	4½× 9	3¾× 8¼	3	10.7	Hand make-up
23	4½× 9	3¾× 8¼	3	9.9	Machine make-up
37	4½× 9	3¾× 8¼	3	10.1	Machine make-up
3	4⅞× 8¾	4¼× 8	2½	10.7	Hand make-up
9	4⅞× 8¾	4¼× 8	2½	10.1	Hand make-up
19	4⅞× 8¾	4¼× 8	2½	10.7	Hand make-up
25	4⅞× 8¾	4¼× 8	2½	10.2	Machine make-up
39	4⅞× 8¾	4¼× 8	2½	10.0	Machine make-up
4	4¾×11¼	4⅛×10¾	3	10.0	Hand make-up
10	4¾×11¼	4⅛×10¾	3	10.1	Hand make-up
20	4¾×11¼	4⅛×10¾	3	10.5	Hand make-up
26	4¾×11¼	4⅛×10¾	3	9.5	Machine make-up
40	4¾×11¼	4⅛×10¾	3	10.0	Machine make-up
11	4¼× 8½	3½× 8¼	2⅜	10.9	Hand make-up
14	4¼× 8½	3½× 8¼	2⅜	10.8	Hand make-up
21	4¼× 8½	3½× 8¼	2⅜	10.8	Hand make-up
30	4¼× 8½	3½× 8¼	2⅜	10.4	Machine make-up
34	4¼× 8½	3½× 8¼	2⅜	10.1	Machine make-up
41	4¼× 8½	3½× 8¼	2⅜	9.5	Machine make-up
6	4⅛× 9¾	3½× 9¼	2⅞	10.8	Hand make-up
12	4⅛× 9¾	3½× 9¼	2⅞	10.6	Hand make-up
22	4⅛× 9¾	3½× 9¼	2⅞	10.4	Hand make-up
28	4⅛× 9¾	3½× 9¼	2⅞	10.4	Machine make-up
42	4⅛× 9¾	3½× 9¼	2⅞	10.2	Machine make-up
13	4½×12	3¾×11½	2¾	10.7	Hand make-up
33	4½×12	3¾×11½	2¾	10.0	Machine make-up
16	4½×10	3¾× 9½	2¾	10.5	Hand make-up
32	4½×10	3¾× 9½	2¾	10.2	Hand make-up
36	4½×10	3¾× 9½	2¾	10.1	Machine make-up

Realizing that proof time would greatly affect the unit volume, the loaves were proofed to a definite height (considered normal) above the top of the pan. The sponge and dough method of fermentation was used and the formula was as follows:

Sponge: %
    Flour 60
    Water (approx.). 62   (Sp. abs.)
    Yeast . . . . 1.75 (Total)
    Malt extract 1   (60° Lintner)
    Yeast food 0.25
Dough:
    Flour 40
    Water (approx.)   63   (Total abs.)
    Salt 2
    Corn sugar 6
    Milk solids 4
    Shortening 3.5

The same flour was used for all of these tests.

Experiments also have been made using different flours and the same pan (top inside 10 in. × 4¼ in., bottom outside 9½ in. × 3¾ in., depth 2¾ in.). The factor 5.9 was used as before. The quality of the flours varied from fairly good to excellent, and hand make-up was employed. The volumes on the resulting bread varied from 9.9 to 10.9 cubic inches per ounce.

The most recent work on commercial pan sizes and shapes was done by Cathcart et al. (1938, 1939).[1] This research determined the best pan shape and size for a 20-ounce loaf of twist bread and round-top bread. All of the experiments were handled on a commercial scale, were machine molded, and were completely scored. Thirty experiments on round-top bread made in the optimum pan (using optimum scaling weights and normal proof) yielded volumes from 9.0 to 9.9 cu. in. per oz. of bread. A similar series on twist bread showed a variation in volume from 9.3 to 10.3 cu. in. per oz. of bread.

3. *Loaves of bread which score highest have a volume of about 10 cu. in. per oz.* Pirrie (1936) has stated that " in the opinion of at least one service agency with national contact in this respect, bread with a unit volume of 10 or a loaf volume of 160 cubic inches for bread weighing one pound, averages the highest score."

Cathcart, Walmsley, and Habenicht (1938) found that, on the average, loaves with a volume nearest to 10 cu. in. per oz. of bread scored the highest.

In order to test this further, experiments were made with a pan (top inside 10⅝ × 5, bottom outside 10⅛ × 4⅜, depth 2¾ in.) that produced optimum results with both twist and round-top bread (Cathcart et al. 1938, 1939). The optimum amount of dough (handled same as Cathcart and co-workers) was scaled into pans of this size and the dough proofed for various lengths of time to vary the unit volume. The

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[1] The optimum number of cubic inches of pan volume per ounce of dough is given by Cathcart and co-workers. The figure is different for each pan depth: 5.99, 5.46, and 5.30 cu. in. per oz. of dough for the 3 in., 2¾ in. and 2½ in. depth respectively. These data were not available when the factor 5.9 was used for experiments reported in Table I.

loaves were then completely scored. Typical results for round-top bread are given in Table II and for twist bread in Table III.

TABLE II
ROUND-TOP BREAD MADE IN OPTIMUM PAN WITH OPTIMUM SCALING WEIGHT—PROOF TIME VARIED

Experiment No.	312	313	314	315	316	317	318	319
Volume (cu. in. per oz.)	7.5	8.8	9.5	10.0	10.0	9.5	10.9	11.1
Minutes proof	40	45	50	55	60	65	70	75
Total score	83.5	87.3	89.0	91.5	92.5	90.5	87.1	83.9
Eating quality	Fair	Fair	Good	Best	Good	Fair	Poor	Poor

TABLE III
TWIST BREAD MADE IN OPTIMUM PAN WITH OPTIMUM SCALING WEIGHT—PROOF TIME VARIED

Experiment No.	304	305	306	307	308	309	310	311
Volume (cu. in. per oz.)	9.0	9.3	9.5	10.0	10.2	9.9	10.6	10.8
Minutes proof	40	45	50	55	60	65	70	75
Total score	91.5	91.7	92.0	92.5	92.3	92.4	91.9	90.7
Eating quality	Good	Good	Good	Best	Good	Fair	Fair	Fair

It will be noted from Tables II and III that the loaves having optimum "Total Score" and "Eating Quality" have a volume of 10. Considering all of the experiments, volumes from about 9.3 to 10.3 produced good-quality bread.

Discussion

From the above it is seen that the optimum volume is very close to 10 cubic inches per ounce of bread and that commercial bread can have too large a volume as well as too small a volume.

It is realized that the optimum loaf volume varies from territory to territory, in accordance with prevailing desires. However, it has been found that too large or too small loaves generally suffer on other points in the bread score, especially on internal characteristics (grain, color of crumb, and texture). Again, when one has to judge bread from all parts of the country, it is a practical impossibility for one to have standards for every section of the country; not only do desired volumes vary within a state, but many times within cities. Lastly, what use is a bread score, anyway? It is not to show how nearly perfect a loaf is, but to serve as a guide to how the baker's bread is today as compared to yesterday, last week, etc. Thus, a standard of 10 will serve very nicely for the whole country, and from experience in these laboratories, it will do it with less error than attempting to set up standards (either in one's mind or on paper) for various sections of the country.

Any value for volume up to 10, measured in cubic inches per ounce of bread, can be recorded directly on the score. Any volume above 10 can be recorded full value (10) on the score, or *better* be degraded by the same amount that it is over 10.

However, a scale which may be followed is given in Table IV.

TABLE IV

VOLUME AND SCORE

Cubic inches per ounce bread by measurement	Value given on score
11.5 to 12.4	8
10.5 to 11.4	9
9.5 to 10.4	10
8.5 to 9.4	9
7.5 to 8.4	8
6.5 to 7.4	7
5.5 to 6.4	6

Experimental Bread Scoring

Although very few data are available, it seems that experimental bread scoring might be handled in the same way as commercial bread scoring with one modification. Saunders *et al.* (1922), Larmour (1929), and others have indicated that the value for loaf volume should represent 40% to 60% of the experimental score; thus it is necessary to multiply the cubic inches per ounce of bread by 4 if the volume is to represent 40% of the total score, etc. The average volume for 25 tests on five different high-grade patent flours was 9.2 cu. in. per oz. of bread made in both the tall and low form pans according to A. A. C. C. procedure.

Experimental loaves baked according to the procedure used at the Institute,[2] yielded an average volume of 9.6 cu. in. per oz. of bread when high-grade patent flour was used.

Summary

Evidence is presented which indicates that unit volume (cubic inches per ounce of bread) can be used directly on a commercial bread score, and that a definite multiple of it can be used on an experimental score.

Acknowledgment

[2] Pan size was: top inside 3½ in. × 6 in., bottom outside 3 in. × 5½ in., and depth 3 in. The amount of dough (very lean formula) scaled in these pans was approximately 325 g. (some variation due to varying scaled weight with flour absorption).

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BETA-AMYLASE ACTIVITY AND ITS DETERMINATION IN GERMINATED AND UNGERMINATED CEREALS [1]

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It is generally recognized that the hydrolytic action of malt on soluble starch results in the production of sugars. The amylase component of malt which can readily bring about this sugar production has been designated the saccharogenic amylase or beta-amylase. Logically therefore this type of amylase activity has been measured by determining the amount of reducing substances produced in a given time or conversely the amount of starch converted into reducing substances. It is recognized that these reducing substances are frequently a mixture of dextrins, glucose, and maltose. However, because of the predominance of maltose in the reaction products the hydrolysis is usually expressed arbitrarily in terms of maltose production.

Most investigators have recognized that the dextrinogenic or alpha component of malt amylase likewise has a degree of saccharogenic activity. Lack of an adequate method for distinguishing between the

saccharifying activities of the two components has resulted in conclusions such as the rather indefinite one that "the contribution made by the alpha-amylase is probably relatively small" (Sallans and Anderson, 1938, p. 408). It was with the purpose of devising a method for the accurate determination of the relative roles of alpha- and beta-amylase in saccharification that the investigation herewith reported was undertaken.

Methods for Determining Beta-Amylase Activity

Two methods of approach have been used in attempts to differentiate between the saccharifying activities of the two malt amylase components. The one most generally adopted has been the differential destruction of the alpha component by such technique as that of Ohlsson (1926, 1930). This has not proved entirely satisfactory and as yet is far from quantitative.

Blom, Bak, and Braae (1937) suggested that the saccharifying actions of alpha- and beta-amylase may be differentiated on the basis of a time-temperature inactivation curve for the malt extract. By progressive heating past the point at which complete destruction of the beta-amylase was effected they found a straight-line relationship between time of heating and saccharifying activity of the remaining alpha component   By extrapolation to zero time of heating the alpha-saccharogenic activity of the unheated extract was determined. Subtraction of this value from the saccharifying activity of the original extract resulted in a value which they postulated as due to beta-saccharifying activity. To those familiar with partial heat inactivation of malt extracts this suggested method has obvious flaws from the standpoint of its application in routine quantitative procedure. It is well known that the sensitivity of alpha-amylase to high temperatures is dependent upon a number of subsidiary factors, among which are extent of dilution, reaction of the extract, and the presence and concentration of certain ions. Too, the above method does not have the speed and simplicity desirable in a routine technique.

An ideal method for differential determination of the saccharifying activities of the two components would require no destructive technique and would have the simplicity and reliability necessary for routine procedure. The method previously devised by Sandstedt, Kneen, and Blish (1939) for the determination of alpha-dextrinogenic activity in the presence of beta-amylase and the reports of several authors that alpha- and beta-amylase are roughly additive in saccharification suggested that this might be possible and led to the development of the proposed method.

Method of Measuring Saccharification

In order to investigate the differential activities of the malt amylases a standard set of conditions for saccharification was necessary. The conditions finally adopted and followed throughout the investigation herein reported were as follows: to 20 cc. of 2% buffered soluble starch solution [2] in a 100-cc. Erlenmeyer flask is added sufficient water so that the final enzyme addition brings the total volume to 30 cc. When the flask contents have come to temperature in the 30° C. bath the enzyme is added and the hydrolysis allowed to proceed for exactly 15 minutes. At the end of the 15 minutes the reaction is stopped by the addition of 20 cc. of 1% sulfuric acid from a fast-flowing pipette As the acid is added the reaction mixture is agitated for rapid mixing. Shortly following the acid addition a 5-cc. aliquot of the solution is transferred to a test tube containing 10 cc. of 0.10N ferricyanide solution and reducing value determined by the method of Blish and Sandstedt (1933) as modified for 0.10N reagents by Sandstedt (1937).

The above technique has several advantages. The short period of hydrolysis permits great rapidity of determination; by starting the runs at two-minute intervals eight hydrolyses may be completed with ease in 30 minutes of total elapsed time. The addition of the sulfuric acid not only terminates the hydrolysis abruptly, but likewise its presence appears to have an as yet inexplicable stabilizing influence on the relationship between maltose present and volume of ferricyanide reduced.

Conversion of Ferricyanide Reduced to Maltose Equivalents

It is recognized that the reducing substance resulting from the action of malt extracts on starch may not be exclusively maltose. However the reducing products have been almost universally reported as maltose equivalents. For convenience and lack of a more suitable designation that practice was followed in the experiments herein reported. It became necessary therefore to construct a table to convert cc. of ferricyanide reduced under the prescribed conditions to mg. maltose. Table I is the conversion table set up for this purpose and may be used as a standard when the experimental conditions are as stipulated. This table was prepared by using an especially prepared sample of maltose of exceptional purity. Conditions characteristic of the experimental technique, such as the buffer and sulfuric acid concentration, were necessarily adjusted to an equivalent basis. It should be emphasized that the conversion data of Table I may be used

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[2] Buffered soluble starch solution: Prepare a suspension of 10 g. (dry weight) of Merck's Lintner soluble starch and pour slowly into boiling water. Cool, add 25 cc. of buffer solution, bring to 500 cc. volume with water and saturate with toluol. The stock buffer solution is 120 cc. glacial acetic acid and 164 g. anhydrous sodium acetate made up to 1000 cc. with water.

TABLE I

FERRICYANIDE-MALTOSE CONVERSION TABLE FOR THE DETERMINATION
OF MALT-SACCHAROGENIC ACTIVITY

0.10 N ferricyanide reduced	Maltose	0.10 N ferricyanide reduced	Maltose
cc.	*mg.*	*cc.*	*mg.*
0.50	1.46	5.50	16.57
1.00	2.92	6.00	18.20
1.50	4.38	6.50	19.85
2.00	5.85	7.00	21.50
2.50	7.32	7.50	23.15
3.00	8.79	8.00	24.88
3.50	10.28	8.50	26.70
4.00	11.83	9.00	28.65
4.50	13.40	9.50	30.65
5.00	14.97	—	—

only when the above experimental conditions are satisfied. Other conditions such as those involving a change in kind or concentration of buffer or the absence of sulfuric acid would necessitate a different conversion table.

To conserve space the data of Table I are presented in abbreviated form. It is suggested that for routine laboratory use the data be plotted on large graph paper from which the maltose equivalents may be read with ease. It should be noted that the maltose corresponding to a found volume of ferricyanide reduced is the amount present in the 5-cc. aliquot taken for reduction. For example if the titration indicates 5.00 cc. of ferricyanide reduced the 5-cc. aliquot contains 14.97 mg. of maltose. The total amount of maltose produced, the amount in 50 cc. total volume, was then 10 × 14.97 or 149.7 mg. If it is desired to express this as starch the conventional factor of 0.95 is used to give 142.3 mg. starch converted to maltose in 15 minutes.

Relationship between Alpha-Dextrinogenic and Alpha-Saccharogenic Activity

Sandstedt, Kneen, and Blish (1939) demonstrated that alpha-dextrinogenic activity could be accurately measured in the presence of beta-amylase. It has proved possible to transpose such quantitative dextrinogenic values into equivalent saccharogenic activity; *i.e.*, from a given dextrinization value the apparent saccharification that this amount of alpha-amylase would produce may be calculated.

The data relating alpha-dextrinogenic activity to alpha-saccharogenic activity are presented in Table II. The dextrinogenic activity is given in reciprocal minutes, the saccharifying activity in mg. maltose. A value listed under reciprocal minutes is that found for a given

TABLE II
RELATIONSHIP OF SACCHAROGENIC ACTIVITY TO THE DEXTRINOGENIC ACTIVITY OF ALPHA-AMYLASE

Alpha-dextrinogenic activity	Alpha-saccharogenic activity	Alpha-dextrinogenic activity	Alpha-saccharogenic activity
1/min.	*mg. maltose*	*1/min.*	*mg. maltose*
0.010	3.17	0.160	46.93
0.020	6.34	0.170	49.39
0.030	9.49	0.180	51.80
0.040	12.66	0.190	54.20
0.050	15.83	0.200	56.58
0.060	18.93	0.210	58.89
0.070	22.01	0.220	61.17
0.080	25.02	0.230	63.37
0.090	27.93	0.240	65.48
0.100	30.74	0.250	67.50
0.110	33.52	0.260	69.42
0.120	36.31	0.270	71.33
0.130	39.05	0.280	73.25
0.140	41.74	0.290	75.17
0.150	44.38	0.300	77.02

amount of alpha-amylase when dextrinizing activity is determined by the technique of Sandstedt, Kneen, and Blish (1939) using an excess of beta-amylase. The corresponding value given for mg. maltose is the apparent maltose produced in 15 minutes by an equal amount of alpha-amylase when it saccharifies starch under the prescribed conditions. For example if 0.05 gram equivalent of alpha-amylase indicates a dextrinizing activity of 0.10 reciprocal minutes (10 minutes), 0.05 gram equivalent would give an apparent saccharification of 30.7 mg. maltose.

The data presented in Table II were established by repeated runs with several samples of alpha-amylase prepared from barley and wheat malts by the Ohlsson (1930) technique. As far as could be determined the preparations were free from beta-amylase activity. For instance prolonged heating of the extracts at 70° C. in an attempt to inactivate any possible remaining traces of beta-amylase resulted in no significant change in the relationships recorded in Table II.

The Saccharifying Activity of Alpha- and Beta-Amylases, Individually and in Combination

In order to compare the saccharifying activity of two enzymes or of more than one concentration of the same enzyme it is important that certain limitations be imposed upon the degree of saccharification permitted. For instance the fact that extracts of ungerminated grains tend to reach a common level of starch hydrolysis at about 60% conversion necessitates a saccharification limit of not more than this

figure. An attempt was made to more clearly define these limits. The data are presented in Figures 1 and 2.

Figure 1 illustrates the progress of starch hydrolysis under the influence of increasing concentrations of beta- and alpha-amylases acting independently. Two beta-amylase extracts were used, one from ungerminated barley the other from ungerminated wheat. Similarly two samples of alpha-amylase, one prepared from barley malt and the other from wheat malt, were used. The differences in activity between the barley and wheat extracts have no general significance, being
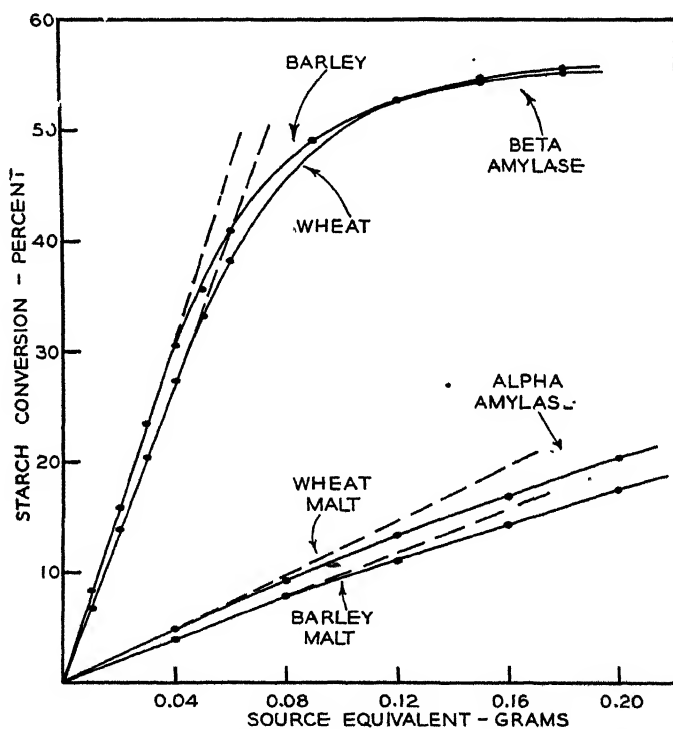
Fig. 1. Relationship of individual amylase concentration to starch saccharification.

characteristic of the individual samples only. The solid lines conform to the found data. The broken lines represent the theoretical progress, assuming perfect linearity.

The curves of Figure 1 illustrate that, under the conditions of the experiment, the relationship between beta-amylase concentration and conversion of the starch to maltose is linear up to approximately 30% conversion. It is apparent then that when determining the saccharifying activity of ungerminated wheat or barley the enzyme concentration

should be so regulated that this limit of 30% is not exceeded in the 15-minute run. The curves of Figure 1 relating alpha-amylase concentration to apparent saccharification indicate that there is a lack of linearity over the whole range. Since saccharification is rarely used as a measure of alpha-amylase activity this does not present a serious problem.

The curves of Figure 2 illustrate the relationship between con-
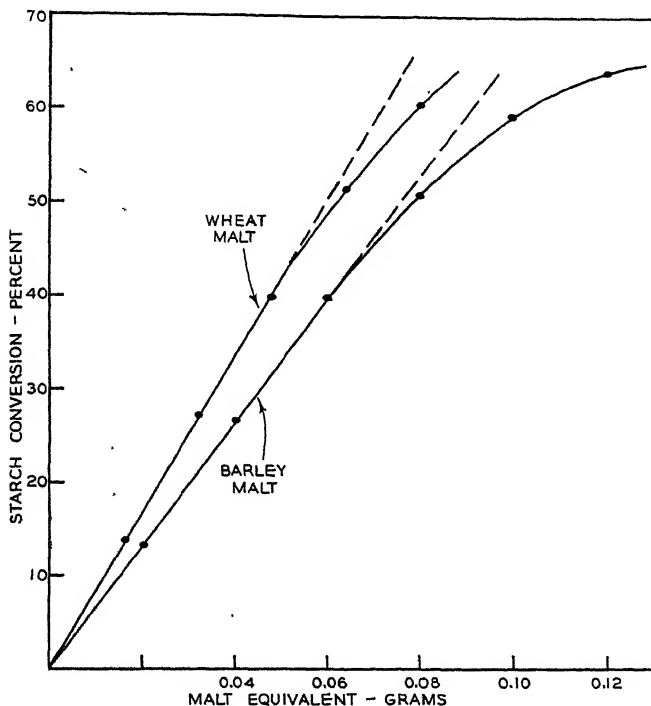
Fig. 2. Relationship of malt concentration to starch saccharification.

centration of unmodified malt and conversion of starch to maltose. Here too the broken lines are extensions of the previous linear relationship and as with the data of Figure 1 the difference in activity between wheat and barley malt is not characteristic of all such malts.

The data recorded in Figure 2 demonstrate that a linear relationship holds only up to the point where approximately 40% of the starch has been converted to maltose.[3] When determining the saccharifying activity of malt extracts it is then important that conversion be not carried past this limiting value.

[3] The data therefore conform to Kjeldahl's law of proportionality. Apparently this limitation has been neglected in some of the more recent researches on malt amylase.

The Additive Nature of Alpha- and Beta-Amylase in Saccharification

It was demonstrated above that when alpha-amylase is caused to saccharify starch in the absence of beta-amylase the apparent saccharification may be calculated from predetermined alpha-dextrinization values. Now if alpha- and beta-amylase should prove to be additive in saccharification, that is if the saccharification produced by a mixture of the two amylases should coincide with the summation of the independent actions, then by subtraction the saccharification due to beta-amylase alone could be calculated.

Ohlsson (1930) and Ohlsson and Uddenberg (1933) found that alpha- and beta-amylases were roughly additive in their action on soluble starch. In further work Stenstam, Björling, and Ohlsson (1934) found that this additive effect was true only for certain ratios of alpha- to beta-amylase. In a more adequate treatment Freeman and Hopkins (1936) reported that the starch-saccharifying activities of the two malt amylase components were additive at low enzyme concentrations but not at high concentrations nor when the beta-amylase concentration was high relative to alpha. Hills and Bailey (1938) reported that, when using purified enzymes, "the amount of maltose produced by alpha- and beta-amylases acting together was approximately the sum of the two amounts produced by their acting separately."

For investigation of the possible quantitative nature of the additive relationship of alpha- and beta-amylase in saccharification a number of determinations were made covering a wide range both of total enzyme concentration and of the ratios of beta- to alpha-amylase. Maltose produced by the independent actions and likewise by the combined action of the two components added simultaneously was measured. The alpha-amylase preparations were obtained from both barley and wheat malts by the Ohlsson (1930) technique and showed no measurable beta-amylase activity. The beta-amylase preparations were hard winter wheat extracts of determined freedom from alpha-amylase.

The data dealing with the additive nature are presented in Table III. The values are arranged in descending order of magnitude of the ratio of beta- to alpha-amylase. These ratios range from one in which the saccharifying activity of beta-amylase was more than 50 times that of alpha to a ratio in which the alpha-amylase activity was more than 20 times that of beta. The range of total concentrations covered varied from a high of 166 mg. maltose produced (39.4% starch conversion) to a low of 21 mg. maltose (4.99% conversion). All activity data are expressed as mg. maltose produced in 15 minutes under the specified conditions.

Table III lists "found" data for the saccharifying activities of the

TABLE III

RELATIONSHIP OF THE RATIO OF ALPHA- AND BETA-AMYLASE IN COMBINATION TO
THE ADDITIVE NATURE OF THEIR ACTIVITIES

Alpha-amylase activity	Beta-amylase activity	Ratio: beta to alpha	Alpha plus beta: found	Alpha plus beta: calculated	Ratio: found to calculated	Deviation: found from calculated
mg. maltose	mg. maltose		mg. maltose	mg. maltose		%
3.0	151.0	50.30	155.5	154.0	1.010	0.97
4.0	155.0	38.75	166.0	159.0	1.044	4.40
3.0	81.0	27.00	83.0	84.0	0.988	1.19
4.0	89.0	22.25	94.0	93.0	1.011	1.08
3.0	44.5	14.83	46.0	47.5	0.968	3.16
9.0	128.0	14.23	139.0	137.0	1.015	1.46
4.0	53.5	13.38	58.5	57.5	1.017	1.74
9.0	83.5	9.28	93.0	92.5	1.005	0.54
13.0	89.0	6.85	103.5	102.0	1.015	1.47
4.0	27.0	6.75	30.0	31.0	0.968	3.23
9.0	60.6	6.73	69.0	69.6	0.991	0.86
3.0	16.5	5.50	21.0	19.5	1.077	7.69
9.0	49.2	5.47	57.0	58.2	0.979	2.06
19.0	97.0	5.11	119.0	116.0	1.026	2.59
27.0	128.0	4.74	153.0	155.0	0.987	1.29
9.0	37.8	4.20	45.5	46.8	0.972	2.78
13.0	53.5	4.12	68.0	66.5	1.023	2.26
25.0	89.0	3.56	114.5	114.0	1.004	0.44
18.0	60.6	3.37	79.0	78.6	1.005	0.51
18.5	62.0	3.35	81.0	80.5	1.006	0.62
27.0	83.5	3.09	109.0	110.5	0.986	1.36
9.0	26.5	2.95	37.0	35.5	1.042	4.23
9.0	25.1	2.79	35.5	34.1	1.042	4.11
18.0	49.2	2.73	67.0	67.2	0.997	0.30
19.0	43.0	2.26	62.0	62.0	1.000	0.00
25.0	53.5	2.14	79.5	78.5	1.013	1.27
18.0	37.8	2.10	54.0	55.8	0.968	3.23
13.0	27.0	2.08	43.0	40.0	1.075	7.50
Av.					1.008	2.23
49.0	97.0	1.980	137.0	146.0	0.938	6.17
45.0	83.5	1.855	121.0	128.5	0.941	5.84
51.5	85.0	1.651	132.0	136.5	0.967	3.30
9.0	12.9	1.433	23.0	21.9	1.050	5.02
18.0	25.1	1.394	45.0	43.1	1.044	4.41
25.0	27.0	1.080	51.0	52.0	0.981	1.92
27.0	26.5	0.981	57.0	53.5	1.065	6.54
49.0	43.0	0.877	87.0	92.0	0.946	5.44
18.0	12.9	0.717	31.0	30.9	1.003	0.32
45.0	26.5	0.589	71.0	71.5	0.993	0.70
51.5	25.5	0.495	82.0	77.0	1.065	6.49
51.5	14.5	0.282	71.0	66.0	1.076	7.57
94.5	25.5	0.270	112.5	119.0	0.945	5.46
94.5	14.5	0.154	108.0	109.0	0.991	0.92
51.5	4.5	0.087	58.0	56.0	1.036	3.57
94.5	4.5	0.048	99.0	99.0	1.000	0.00
Av.					1.003	3.98
Av. for all determinations					1.006	2.86

enzymes individually and in combination. The summations of the two individual activities are listed as "calculated" activities. Two methods of comparing the "found" and "calculated" results are used. The data for the ratio of found to calculated express the degree to which the two values satisfy a one-to-one ratio; those for the deviation between found and calculated values indicate the magnitude of error which might be anticipated in the application of such technique. It should be emphasized that because of the nature of the operations necessary to obtain the data of Table III the probability of error is greater than would be anticipated in actual practice. The 44 runs are divided into two groups: 28 runs in which the ratios of beta-amylase activity to alpha-activity are above 2.00, and the remaining 16 in which this ratio is below 2.00. Average ratios and percent deviations between "found" and "calculated" data are given for each group as well as for all 44 runs.

The data indicate that there is no consistent deviation from a one-to-one ratio of the found and calculated results; the average ratio for all 44 runs was 1.006. The percentage of deviation between the two values is not serious throughout but does appear to be somewhat less for the group in which beta-amylase is high relative to alpha. The average deviation for the 28 runs in which the ratio of beta- to alpha-amylase was above 2.00 is 2.23% as compared to 3.98% for those ratios below 2.00.

Using the amount of maltose produced in the "found" column as a measure of the enzyme concentration, the data of Table III may be used to illustrate the relationship of total concentration to the additive nature of the two components. In Table IV the data for the 28 runs where the ratio of beta- to alpha-amylase was greater than 2.00 are arranged into three groups: (1) eight combinations from which the maltose produced was above 100 mg., (2) ten combinations from which the maltose produced was between 100 and 60 mg., and (3) ten from which the maltose produced was less than 60 mg.

It is apparent from Table IV that the closest agreement between the found data and the calculated is in the intermediate group. Here the average percentage of deviation is less than one, as compared to 1.75% deviation for the high and 3.97% for the low group. However it is evident that relatively high concentrations of the enzymes do not destroy the relationship as indicated by the reasonably low error of 1.75% in the high group. The average deviation of approximately 4% for the group of low concentrations appears more serious. However this would seem to be nothing more than an indication that measurements of low degrees of saccharification are subject to a relatively greater percentage of error than measurements made at higher levels.

TABLE IV

RELATIONSHIP OF TOTAL ENZYME CONCENTRATION TO THE ADDITIVE NATURE OF ALPHA- AND BETA-AMYLASE ACTIVITY

Group (1): maltose above 100 mg.		Group (2): maltose between 60 and 100 mg.		Group (3): maltose below 60 mg.	
Maltose produced	Deviation: found from calculated	Maltose produced	Deviation: found from calculated	Maltose produced	Deviation: found from calculated
mg.	*%*	*mg.*	*%*	*mg.*	*%*
166.0	4.40	94.0	1.08	58.5	1.74
155.5	0.97	93.0	0.54	57.0	2.06
153.0	1.29	83.0	1.19	54.0	3.23
139.0	1.46	81.0	0.62	46.0	3.16
119.0	2.59	79.5	1.27	45.5	2.78
114.5	0.44	79.0	0.51	43.0	7.50
109.0	1.36	69.0	0.86	37.0	4.23
103.5	1.47	68.0	2.26	35.5	4.11
—	—	67.0	0.30	30.0	3.23
—	—	62.0	0.00	21.0	7.69
Av.	1.75	Av.	0.86	Av.	3.97

The data of Table III permit the conclusion that alpha- and beta-amylase are additive in saccharification throughout a wide range of ratios of beta to alpha, and particularly so when the concentration of beta-amylase is high relative to alpha. In addition, as arranged in Table IV, the data illustrate that, at least below the saccharification level represented by 40% starch conversion, total concentration of the enzymes does not materially affect the additive relationship.

The Determination of Beta-Amylase Activity

Data presented above indicate that alpha- and beta-amylase are additive in saccharification and that the saccharifying activity of alpha-amylase may be readily calculated from the determination of its absolute dextrinizing activity: This dextrinizing activity may be determined by the routine procedure of Sandstedt, Kneen, and Blish (1939). It becomes then a simple matter to determine the absolute beta-saccharogenic activity of a malt extract.

The steps in the determination of beta-amylase activity follow in order:

1. Determine the minutes required for dextrinization when using the alpha-amylase method of Sandstedt, Kneen and Blish (1939).

2. Using the method for determining saccharification outlined above find the total mg. of maltose produced in 15 minutes. For this determination the precaution must be taken that the concentration of malt used does not give greater starch hydrolysis than the limiting 40% conversion.[4]

[4] Malt concentrations which may be used with a resultant starch conversion safely below the 40% level vary from the equivalent of 0.01 g. for malts of very high diastatic power to 0.05 g. for low diastatic malts.

3. Calculate in reciprocal minutes the dextrinization time for the amount of alpha-amylase present in the malt sample used for saccharification. This value may be found by multiplying the reciprocal of the time in minutes found in the alpha-amylase determination by the factor:

$$\frac{\text{Malt equivalent used for saccharification (g.)}}{\text{Malt equivalent used for alpha-dextrinization (g.)}}$$

If the alpha-amylase activity is already recorded as the alpha-amylase units of Sandstedt, Kneen, and Blish (1939) the desired reciprocal minutes for the amount of alpha-amylase acting in saccharification may be derived by multiplying the figure for alpha-amylase units by the factor:

$$\frac{\text{Malt equivalent used for saccharification (g.)}}{0.4 \times 60}$$

4. Using Table II find the mg. maltose corresponding to the reciprocal minute value for the alpha-amylase taking part in saccharification. Subtract this value from the total mg. of maltose produced in the 15-minute saccharification. The difference represents the mg. of maltose produced by the beta-amylase present in the malt sample.

5. Beta-amylase units may now be calculated as the number of grams of starch converted to maltose by the beta-amylase of one gram of malt in one hour at 30° C.

The application of the above technique to data found for a barley-malt sample perhaps best illustrates the procedure. The pertinent data for one malt follow:

Dry matter content.. .... 93.07%
Alpha-dextrinization time for 0.05 g. equivalent........ 14.0 min.
Saccharification by 0.03 g. equivalent: ferricyanide re-
    duced by 5 cc. aliquot less starch blank............. 4.26 cc.

Using these data the following calculations are made:

Maltose produced by 0.03 g. equivalent (Table I).. 126.5 mg.

Alpha-dextrinization time for 0.03 g. equivalent.... $\frac{0.03}{0.05} \times \frac{1}{14} = 0.0429$ reciprocal min.

Maltose due to alpha-amylase activity (Table II). 13.6 mg.
Maltose due to beta-amylase activity......... .. $126.5 - 13.6 = 112.9$ mg.
Starch converted to maltose by beta-amylase..... $112.9 \times 0.95 = 107.3$ mg.

Starch conversion in one hour.................. $0.1073 \times \frac{60}{15} = 0.429$ g.

Dry weight of malt equivalent used for saccharifi-

cation....................... .... $\frac{93.07}{100} \times 0.03 = 0.0279$ g.

Starch converted to maltose by the beta-amylase of
    one gram of malt in one hour at 30° C. (beta-

amylase units).... .... $\frac{0.429}{0.0279} = 15.38$ g.

That is, the malt has a beta-amylase activity of 15.4 units.

Definition of " Units "

In the past the arbitrary calculations commonly used to express saccharogenic activity, such as "degrees Lintner," have been meaningless from the standpoint of any true definition of the nature and extent of the hydrolysis taking place. Sandstedt, Kneen, and Blish (1939) suggested that the logical terminology for defining alpha-amylase activity is in terms of grams of starch dextrinized by one gram of malt in one hour. There is no logical reason why saccharogenic activity should not be expressed in a similar manner. Accordingly the following uniform terminology has been adopted and used throughout this communication where absolute and comparative definition of amylase activity is desired. The definition for alpha-dextrinogenic activity is included for comparison.

Malt-saccharogenic units.—The number of grams of soluble starch converted to maltose by one gram of malt in one hour at 30° C.

Beta-saccharogenic units (beta-amylase units).—The number of grams of soluble starch converted to maltose by the beta-amylase of one gram of malt in one hour at 30° C.

Alpha-dextrinogenic units (alpha-amylase units).—The number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 30° C.

The above unit values are calculated on the dry-weight basis throughout. The specifications as outlined for each determination must be followed exactly. Too, it should be emphasized again that a limit of 40% starch conversion should not be exceeded in the malt saccharification and 30% in the case of saccharification by the beta-amylase of ungerminated cereals such as barley and wheat.

Application of the Beta-Amylase Method

A group of five barley malt grains was selected to demonstrate the application of the beta-amylase method. These grains were ground finely in a burr mill and one gram of meal extracted with 100 cc. of water for one hour at 30° C. The extracts were centrifuged for five minutes and then poured rapidly through cotton plugs. Five-cc. aliquots of the extracts (equivalent to 0.05 g. malt) were used for the determination of alpha-amylase, the range of dextrinization times being from 14.0 to 22.25 minutes. For saccharification 3-cc. aliquots (equivalent to 0.03 g. malt) were used and gave a range of hydrolysis from 9.63% to 30.05% starch conversion. The results obtained are given in Table V. Data are presented for alpha-dextrinogenic units, malt-saccharogenic units, and for beta-saccharogenic units. In addition the fraction of the saccharification due to beta-amylase alone is illustrated.

TABLE V

STARCH-DEGRADING PROPERTIES OF VARIOUS MALTS

Malt	Alpha dextrinization	Malt saccharification	Beta saccharification	Fraction of saccharification due to beta-amylase
	Units	Units	Units	%
1	36.8	17.21	15.38	89.4
2	35.6	14.25	12.45	87.4
3	25.1	12.20	10.95	89.8
4	41.1	9.02	6.97	77.3
5	23.1	5.50	4.34	78.9

The data of Table V permit several significant conclusions. There is a high degree of correlation between the saccharifying activity of a malt and its beta-amylase content. On the other hand there seems to be no necessary correlation between the alpha-amylase activity and either the saccharifying activity or the beta-amylase activity of a malt. For example malt No. 4 has the highest alpha-amylase activity but is next to the lowest in both saccharification and beta-amylase activity. The alpha-amylase content of a malt obviously influences its saccharifying activity, the degree of this influence varying with different malts. With three of the five malts illustrated more than 10%, and with the remaining two more than 20%, of the saccharification may be attributed to the alpha-amylase content. It would seem that such differential methods as outlined above are essential in any attempt to gain an accurate conception of the roles played by the components of malt amylase in starch saccharification.

Summary

The saccharifying power of a malt extract either unmodified or modified in such a manner as to inactivate most of the alpha-amylase is commonly used as a measure of beta-amylase activity. It is generally recognized that alpha-amylase contributes to malt saccharification, the degree of that contribution being of uncertain magnitude. In order to obtain an accurate determination of the beta-amylase present it is necessary therefore to take cognizance of the presence of alpha-amylase and the extent of its influence on the saccharifying activity.

A rapid technique for measuring saccharifying activity was developed. This procedure involves a 15-minute digestion followed by acidification to halt the enzymic activity. Maltose production is measured by the ferricyanide method. A conversion table relating cc. of ferricyanide reduced to mg. maltose present is provided.

It was found that the saccharogenic activity of alpha-amylase was proportional to its dextrinogenic activity. From absolute alpha-

dextrinogenic values measured by the method of Sandstedt, Kneen, and Blish (1939) and simultaneously run alpha-saccharogenic activities, the relationship between these values was determined. A conversion table relating saccharogenic power to previously determined alpha-dextrinogenic activity is presented.

The limiting percentage of starch conversion below which there exists a linear relationship between enzyme concentration and starch saccharification was determined for the individual amylase components and for unmodified malt extract. For malt extract the relationship is linear up to 40% starch conversion and for beta-amylase alone up to 30% conversion. For alpha-amylase alone there appears to be no definite region of linearity.

The additive nature of alpha- and beta-amylase in saccharification was investigated over a wide range of combinations covering various total concentrations and various ratios of beta- to alpha-amylase. It was demonstrated that, within the limits of the experiment, an additive relationship holds throughout. Combinations in which the beta-amylase was low relative to alpha showed slightly more deviation from this relationship than did other combinations. In combinations where the saccharogenic power of the beta component is more than twice that of the alpha component, a condition generally characteristic of malts, the saccharogenic activity of a mixture of alpha- and beta-amylases is the sum of the individual activities of the two acting independently. The activity of the beta-component of malt may then be determined by subtracting from the total saccharogenic value the previously calculated alpha-saccharogenic value. These calculations may be performed with only slightly less accuracy when the ratio of beta- to alpha-saccharogenesis is less than 2.00.

Unit definitions are suggested for malt-saccharogenic activity and for beta-saccharogenic activity. These units, as were those proposed for alpha-dextrinogenic activity, have as their basis the number of grams of starch hydrolyzed by one gram of malt in one hour at 30° C.

Application of the beta-amylase method to barley malts is discussed. The lack of parallelism between alpha- and beta-amylase contents is demonstrated as well as the variation in the fraction of malt saccharogenic activity attributable to beta-amylase.

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REPORT OF THE 1939–40 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

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The program followed by the 1939–40 Biscuit and Cracker Committee was planned to deal with the three recommendations of the previous committee (Simmons, 1940): (1) That further work of this same type be done to build up a greater volume of data to correlate more closely the laboratory evaluation of cracker flours with their actual shop performance. (2) That further study be made on the scoring of crackers in order to obtain a closer agreement among collaborators. (3) That a statistical study be made of all pertinent data accumulated by the committees in the last three years, this to be done in order that more practical conclusions may be reached.

The statistical study is being prepared and will be given in a separate report. Work done by the last four committees indicates that " The quality of the crackers seemed to depend upon the type of flour used in the sponge rather than upon the dough flour used in the formulae " (Reiman, 1937; Brown, 1938; Simmons, 1939). With this in mind, the committee selected three types of sponge flour and one dough flour.

The chemical analysis of each flour was made by two members of the committee, while the hydrogen-ion concentration was made by one

member.   Viscosity tests were made by each member of the committee, following the methods outlined by Bayfield (1936), procedures No. 1 and No. 2.   On the basis of his own viscosity tests and the analytical data furnished with the samples, each committee member classified the samples.   Bread was baked by one member of the committee, using the regular A. A. C. C. method and a loaf from each flour was sent to each committee member for classification as to use as a sponge and/or dough flour.

Crackers were baked in a commercial shop, following schemes used by Reiman (1938) and Brown (1939) ; *i.e.*, the three sponge flours were baked with the dough flour and in addition the weak sponge flour was used as a dough flour.   Three cracker bakes were made at three-week intervals.   Each member of the committee scored and evaluated the crackers according to the method outlined in *Cereal Chemistry* **15**: 37.   One member of the committee analyzed the baked crackers and another determined the shortometer values.

For the sake of continuity, the flours were designated as Nos. 17, 18, 19, and 20.   Table I gives the analytical data and the type of wheat from which each flour was milled.

TABLE I

ANALYTICAL DATA AND FLOUR TYPE

Sample No.	Protein (15% H_2O)	Ash (15% H_2O)	pH	Maltose [1]	Used for	Wheat used
	%	%	.	mg.		
17	9.19	.406	5.75	157	Strong sponge	Illinois Red
18	8.56	.408	5.83	125	Sponge	Indiana Red
19	8.80	.392	5.81	134	Sponge	Ohio Red
20	7.76	.395	5.80	116	Dough	Indiana Red

[1] Maltose determination made by W. H. Hanson.

Discussion of Results

This year's results showed exceptionally close agreement by the collaborators on their viscosity results, both by the no-time method and the one-hour digestion method.   In only one case was the variation greater than ± 3 degrees from the average.   Since this committee is national, it speaks well for the progress which has been made in the viscosity test, both by the one-hour digestion and the no-time method.   Table II gives the average by both methods.

It should be noted that, although milled from wheat grown in two different states, flours No. 18 and No. 19 showed almost identical viscosities, both by the one-hour and the no-time method.

Table III gives the average individual classification of flours based on the viscosity tests.   The classification was made from the final read-

ings respectively, from both the one-hour and the no-time method. The agreement between collaborators was so close that only the average is shown.

TABLE II

AVERAGE VISCOSITY DETERMINED BY THE ONE-HOUR AND THE NO-TIME METHOD

| | Sample number and method | | | | | | | |
| | 17 | | 18 | | 19 | | 20 | |
	1-hour	No-time	1-hour	No-time	1-hour	No-time	1-hour	No-time
Average	116.6	86.4	92	71.7	92	70	61.8	48.5

TABLE III

AVERAGE CLASSIFICATION OF FLOURS ON BASIS OF ANALYTICAL AND VISCOSITY TESTS

| | Sample number | | | |
Method	17	18	19	20
1-hour	Strong to very strong sponge	Sponge to strong dough	Sponge to strong dough	Dough
No-time	Strong sponge	Sponge to strong dough	Weak sponge to strong dough	Dough

In two cases the flours were rated one degree higher by the one-hour method than by the no-time method. This confirms last year's report (Simmons, 1939) : " The one-hour method places the border-line flours slightly higher than the no-time method."

The baking test classification (Table IV) follows very closely the classification of these flours by the viscosity tests. The classification probably parallels the viscosity results of the one-hour method more closely than the no-time method. There was not the tendency, this

TABLE IV

CLASSIFICATION OF FLOURS BY THE BAKING TEST

| | Sample number | | | |
Collaborator	17	18	19	20
No. 1	Strong sponge	Sponge	Sponge	Strong dough
No. 2	Very strong sponge	Sponge	Sponge	Strong dough
No. 3	Strong sponge	Sponge	Sponge	Dough
No. 4	Sponge	Weak sponge	Weak sponge	Weak dough
No. 5	Strong sponge	Sponge	Sponge or dough	Dough
No. 6	Sponge	Sponge or dough	Sponge or dough	Dough
No. 7	Sponge	Sponge or dough	Sponge or dough	Dough
No. 8	Strong sponge	Medium sponge	Weak sponge or dough	Dough

year, to place the borderline flours a degree higher by the baking test, as was noted in the preceding reports. This may be due to the fact that all of these flours showed low diastatic values. Since this factor was not determined by preceding committees, no conclusions can be drawn. It might be well in future work of this type to incorporate this determination.

Flour No. 19, which showed almost the same viscosity by both methods as No. 18, was rated by the majority as a weak sponge or dough flour by the baking test, while No. 18 was rated as a sponge flour.

The same tendency was noted this year, as to the individual scoring of crackers, that "There is a considerable difference between the way individuals score crackers, both in valuation and their rating."

Table V shows the ranking of the crackers baked from the four

TABLE V

RANKING OF CRACKERS BY SCORES AND SHORTOMETER VALUES

Collab- r orator	First bake Samples				Second bake Samples				Third bake Samples			
	A	B	C	D	2	1	4	3	IV	III	II	I
No. 1	3	2	1	4	1	3	2	4	4	1	3	1
No. 2	4	3	1	2	1	4	2	3	4	3	1	2
No. 3	3	2	4	1	3	4	1	2	3	2	1	4
No. 4	4	1	1	2	2	2	1	2	2	3	1	4
No. 5	4	3	1	2	1	4	3	2	1	3	· 2	4
No. 6	2	1	3	4	4	2	1	3	3	4	2	1
No. 7	3	2	4	1	4	1	3	2	2	3	4	1
No. 8	4	2	1	3	4	3	1	2	3	2	1	4
Total	27	16	16	19	20	23	14	20	22	21	15	21
Rank	4	1	1	2	2	4	1	2	4	2	1	2

	SUMMARY: Samples				SHORTOMETER RANK: Samples			
	A	B	C	D	A	B	C	D
First bake	4	1	1	2	2	3	1	4
Second bake	2	4	1	2	2	3	1	4
Third bake	4	2	1	2	3	1	2	4
Total	10	7	3	6	7	7	4	12
Rank	4	3	1	2	2	2	1	4

flours. Although there is a difference between individuals in evaluation and rating, the total ranking of crackers this year shows close agreement for the three bakes.

The *A* cracker, which ranked fourth on two of the bakes, was baked with the No. 17 flour used as a sponge. This flour was rated by both the baking test and the viscosity by the one-hour digestion method as a "strong sponge" to "very strong sponge" flour. This would confirm the observation made last year (Simmons, 1939), that "Viscosity by

the one hour digestion method more properly classifies this flour than the no time method."

The C cracker, which ranked first on all three bakes, was baked with the No. 19 flour as a sponge flour. That flour was rated as a slightly weaker flour by the baking test than the No. 18 flour which was used as a sponge flour in baking the B cracker. The B cracker ranked third, although placed equal to C on the first bake.

The No. 19 flour was used as both a sponge and a dough flour in the D cracker, and although classed as a sponge or dough flour by the baking test, the ranking of the crackers indicates that it offered fewer problems as a sponge flour.

The results of the cracker bakes tend to further confirm the observations made by Reiman (1937) and Brown (1938), that "The quality of crackers seems to be more dependent upon the type of sponge flour than the type of dough flour used in the formula." Even though the D cracker was baked with a very strong dough flour it rated second.

The shortometer results are not so gratifying as they might have been, except on the B and C crackers, where they agreed with the ranking of crackers by the collaborators. The D cracker rated fourth on all three bakes by the shortometer, while the A cracker ranked fourth by the collaborators and second by the shortometer.

The chemical and physical analyses of the crackers (Table VI), while

TABLE VI

CHEMICAL AND PHYSICAL ANALYSES OF CRACKERS [1]

Bake No.	Cracker	Moisture	Protein (N×6.25)	Ash	Fat	Shortometer value	Av. pH	Av. thickness	Av. count per lb.
		%	%	%	%			In.	
1	A	5.45	9.37	2.21	13.14	51	8.5	3	127
	B	5.85	8.84	2.59	12.93	53	8.55	$2\frac{15}{16}$	117
	C	4.95	9.19	2.71	13.11	48	8.6	$2\frac{14}{16}$	123
	D	5.90	9.63	2.47	13.13	59	8.6	$2\frac{14}{16}$	118
2	A	5.80	9.70	2.22	13.19	59	8.4	$3\frac{2}{16}$	107
	B	7.70	9.30	1.79	12.08	64	7.8	$2\frac{14}{16}$	114
	C	7.00	9.00	2.36	13.14	55	7.8	$2\frac{15}{16}$	118
	D	5.20	9.00	2.49	13.06	65	8.3	3	109
3	A					49	8.6	$3\frac{3}{16}$	114
	B					43	8.4	$2\frac{15}{16}$	123
	C					48	8.3	$2\frac{15}{16}$	123
	D					61	8.3	$3\frac{1}{16}$	115

[1] Chemical analyses by T. E. Hollingshead; shortometer values by H. J. Loving.

not differing greatly from those reported by previous committees, did show some points of variation. The average pH of the cracker seemed to be somewhat higher, as was also the count per pound. There was a

marked difference in the shortometer values which were expressed in 32nds of a pound in all cases. This year the values are between 49 and 65, while last year the values were between 63 and 90, and two years ago Brown's (1938) report showed values between 56 and 80.

Last year the committee observed that "The higher the moisture content the higher the shortometer value." This seemed to hold true in most cases this year.

Conclusions

Although the work done by the committee this year has been essentially a repetition of the work done by previous committees, it is felt that the results have served as a substantial confirmation of the work of these committees on evaluation of flours by the baking and viscosity tests.

A closer agreement has been secured between collaborators on the viscosity tests and on the scoring of baked crackers.

Recommendations

The committee recommends that further work on cracker flours be suspended until the statistical study can be brought up to date. This report is to cover the five years' work including the work of the committee this year.

It is also recommended that work be done on biscuit or cookie type flours in order to bring the testing of this class of flours to the present status of the cracker flours.

Acknowledgments

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REPORT OF THE 1939–40 COMMITTEE ON METHODS OF TESTING SELF–RISING AND PHOSPHATED FLOURS

O. E. GOOKINS, *Chairman*

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(Read at the Annual Meeting, May 1940)

Committees of former years have provided a formula, methods of baking, a scorecard, and definitions and evaluations of score-card terms. A series of photographs representing a range of textures and also a method of preparing semipermanent color standards for biscuit scoring have been presented. Studies on variations due to ovens and effects of varied absorptions have been made. Numerous collaborative bakings, with different types and kinds of flours, have shown considerable variability in scoring. The use and purpose of biscuit-baking tests have been expounded, and attempts to arrive at specifications for biscuit-flour standards have been reported.

By collaborative baking and by questionnaires, this committee endeavored to determine two things: (1) whether baking to a uniform oven loss would be a means of bringing collaborators to a common basis that would result in more uniform biscuit volumes, and (2) specifications for standard flours to be used to smooth out personal, machine, and atmospheric differences in performing the biscuit baking test.

Samples of three self-rising flours, with entirely different characteristics, were sent to each of the collaborators. Sample No. 1 was a hard-wheat patent, 10.5% protein; sample No. 2 a soft-wheat patent, 9% protein; sample No. 3 a soft-wheat cake flour, 7.5% protein. The pH of No. 1 was 5.5; No. 2, 5.4; while No. 3, which was chlorine bleached, had a pH of 4.9. Viscosities of these flours were: No. 1, 229°; No. 2, 98°; No. 3, 27° MacMichael. Ash contents, respectively, of these flours were: No. 1, 0.38%; No. 2, 0.34%; No. 3, 0.32%.

Self-rising ingredients were mixed with these flours, using the formula given in *Cereal Chemistry* by Walter (13:722): flour 100, hydrated monocalcium phosphate 1.875, sodium bicarbonate 1.5, salt 2.0

Specific directions for baking and scoring the biscuits were given. Each collaborator was asked to maintain a 14% oven loss, which was 1% above the average oven losses in the baking tests of the 1938–39 committee. Sufficient flour for three bakes was sent to each collaborator.

Results of the baking tests which were reported by three of the collaborators, as given in Tables I, II, and III, indicated rather definitely the need for a standard flour to be used as a basis of comparison

in order to obtain closer agreement on items of the score card. While the actual scores varied considerably, the relative ranking of the three flours for the various score-card items showed some semblance of agreement. In the tenderness ranking the three reporting collaborators agreed that flour No. 3 was the best. Flavor scores varied consider-

TABLE I

SELF-RISING SAMPLE NO. 1 (HARD PATENT)

Standard score		Collaborators			Average score
		A	B	C	
Grain	10	9.8	9.0 heavy cell	7.0	8.6
Tenderness	10	8.8	8.5	8.5	8.6
Flavor	20	19.8	17.0	17.5	18.1
Crumb color	20	17.0	16.4 creamy	15.0	16.1
Volume [1]	40	41.8	41.6	41.4	41.6
Total	100	96.2	92.5	89.4	93.0
Water used		162 cc.	160 cc.	156 cc.	159 cc.
pH biscuit		Col. 7.15	El. 7.15	Col. 7.0 El. 7.3	7.15
Oven loss [2]		13.9	14.6	15.3	14.6
Spec. volume [3]		2.09	2.08	2.07	2.08
No. bakes		3	3	2	—

[1] $\frac{\text{cc. biscuit}}{\text{g. dough}} \times \frac{40}{2}$.

[2] $\frac{\text{wt. dough} - \text{wt. biscuit}}{\text{wt. dough}}$.

[3] $\frac{\text{cc. biscuit}}{\text{g. dough}}$.

TABLE II

SELF-RISING SAMPLE NO. 2 (SOFT PATENT)

Standard score		Collaborators			Average score
		A	B	C	
Grain	10	10	9.5	8.5	9.3
Tenderness	10	10	9.0	7.0	8.7
Flavor	20	20	19.0	17.5	18.8
Crumb color	20	20	18.3	17.0	18.4
Volume	40	42.2	40.6	40.2	41.0
Total	100	102.2	96.4	90.2	96.2
Water used		156 cc.	148 cc.	154 cc.	153 cc.
pH biscuit		Col. 7.15	El. 7.15	Col. 7.0 El. 7.33	7.15
Oven loss		14.0	14.4	13.63	14.0
Spec. volume		2.11	2.03	2.01	2.05
No. bakes		3	3	2	—

TABLE III

SELF-RISING SAMPLE NO. 3 (SOFT CAKE-FLOUR PATENT)

Standard score		Collaborators			Average score
		A	B	C	
Grain	10	10	8.4 weak, close	9.0	9.1
Tenderness	10	12	10.0 short	9.0	10.3
Flavor	20	19.2	18.5 dry	18.0	18.6
Crumb color	20	22	19.0 dead	18.0	19.7
Volume	40	39	37.6	43.2	39.9
Total	100	102.2	93.5	97.2	97.6
Water used		149 cc.	136 cc.	152 cc.	146 cc.
pH biscuit		Col. 7.05	El. 7.03	Col. 7.0	7.07
				El. 7.26	
Oven loss		15.4	15.2	14.88	15.2
Spec. volume		1.95	1.88	2.16	1.96
No. bakes		3	3	2	—

ably; individual tastes and perhaps the differences in time that elapsed before baking by the different collaborators may have affected these. Color-of-crumb scores ranked the flours in the same order in each case, with flour No. 3 first, No. 2 second, and No. 1 third. Volume scores agreed very well on Nos. 1 and 2. Sample No. 1 ranged from 41.4 to 41.8 and No. 2 from 40.2 to 42.2. On No. 3 the collaborators did not check so well, their scores ranging from 37.6 to 43.2.

The pH of the biscuits checked well, averaging 7.15, 7.15, and 7.07 for Nos. 1, 2, and 3, respectively.

Oven losses were maintained reasonably close to 14%, with a maximum variation of ±1.4% for all collaborators on the three samples. There is probably insufficient collaborative data here to say definitely whether controlled oven loss points toward any better agreement on volume. Samples No. 1 and No. 2 tend to support this supposition, while No. 3 would disprove it. Additional tests in the Quaker Oats laboratory gave similar specific volumes over a range of 5% oven loss. The tendency of the 1938–39 committee's collaborative bakings to show lower specific volumes with lower oven losses was not borne out in the above-mentioned tests.

It is our belief that the kind of mixing and baking equipment used, the technique and experience of the operator, and the volume-measuring device are the three most important items affecting volume values. The time and type of storage of the self-rising flour prior to baking are also factors which affect volume. It is our opinion that controlled oven loss is *not* the answer for better agreement in volume scoring.

The second item in this committee's problem for the year was the determination of specifications for standard flours. Opinions on this were obtained by questionnaires and by reference to previous committees' ideas. The need for a standard or standards has been known for several years. Most suggestions agreed that a short-patent bleached soft-wheat flour of approximately 9% protein, 5.3 to 5.6 pH, and under 0.36 ash, should constitute one standard. The use of supplementary standards, such as a soft straight and a soft clear, and also a hard-wheat short-patent, were suggested. It is the general opinion that the soft-wheat short-patent flour should be one for whose grade the standard score totals 100. The flour should have a good color.

The question was raised by a member of the committee as to whether the recommendation of self-rising flour standards falls within the province of this committee. The problem is complicated by the wide variety of self-rising and phosphated flours on the market today. The writer concurs in this point, that it is impractical to try to set up a definite standard or standards to fit everyone's need. It is suggested that the few specifications given for a soft-wheat patent flour standard be adopted as a guide only, and that in any collaborative baking tests a flour of such type be sent along and specified as the standard to be used in judging the other samples.

In conclusion, this year's committee has, by questionnaire and by collaborative baking tests, first, found that controlled oven loss does not seem to result in more uniform biscuit volumes; second, suggested a few specifications on a soft-wheat short-patent flour to be used as a guide in selecting a standard flour for scoring biscuits, and, further, suggested that in all collaborative biscuit baking a flour of such type be sent and designated as standard.

Acknowledgments

REPORT OF THE 1939-40 COMMITTEE ON METHODS OF TESTING CAKE FLOUR

J. W. Montzheimer, *Chairman*

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(Read at the Annual Meeting, May 1940)

Our program for the past two years has been directed towards a comparison of different cake test formulas and the behavior of various cake flours.

Table I gives percentages of ingredients in the various formulas

TABLE I

PERCENTAGES OF INGREDIENTS USED IN FORMULAS BY 1938–39 CAKE COMMITTEE

Formula	Flour	Sugar	Shortening	Eggs	Milk	Salt	Baking powder
A.A.C.C.	28.89	27.78	7.22	9.11[1]	25.56	0.44	1.0[2]
Modified	28.86	27.74	7.22	9.12[1]	25.51	0.44	1.11
2	27.7	27.7	11.0	9.9[1]	22.0	0.6	1.1
5	26.52	26.52	15.15	17.05[3]	13.45	0.75	0.56
6	26.14	26.14	13.07	13.07[3]	19.60	0.8	1.18[2]
1	25.7	30.6	9.4[4]	14.00[1]	18.0	0.9	1.4
4	24.62	29.55	9.4	14.77[1]	19.70	0.78	1.18
10	23.92	28.70	9.56[4]	14.36[1]	21.54	0.77	1.15
3	21.09	30.00	11.72[4]	12.65[3]	22.48	0.75	1.31
7	21.06	29.49	11.50[4]	15.84[1]	20.25	0.81	1.05

[1] Egg whites.
[2] Used soda and cream of tartar.
[3] Whole eggs.
[4] Special cake shortening.

used. The formulas are grouped according to the percentage of flour contained in the batter, which ranges from 28.89% in our present A.A.C.C. test formula down to 21.06% in the formula at the bottom of the list, which is made with a special cake shortening. It is worthy of note that the percentage of sugar in the batters varies from 26.14% to 30% and that the so-called "hi-ratio" cakes contain only 4% more sugar in the batter than the lean-formula cakes. The really important change in the batter is the reduction of the flour from 5% to 7% when using the special type of shortening.

Special consideration was given by this year's committee to a new scoring method copied after the system being used in the canning industry. This is illustrated in Table II. The outstanding points in this system are:

1. Volume is not included in the score but measured in cubic centimeters.

		Score			
		Excellent	Good	Fair	Poor
Symmetry	15	15–13	12–10	9–6	5 or less
Crust	5	5	4	3	2 or less
Texture					
Tenderness	15	15–13	12–10	9–6	5 or less
Silkiness	15	15–13	12–10	9–6	5 or less
Grain	25	25–22	21–18	17–14	13 or less
Color	15	15–13	12–10	9–6	5 or less
Flavor (taste and eating quality)	10	10–9	8–7	6–5	4 or less

2. Consideration is given to flavor as part of the score.

3. A so-called "stop" system is used. In the "stop" system each unit of the score is divided into four classifications: excellent, good, fair, and poor. A certain percentage of the score is allotted to each classification. A cake to grade excellent would necessarily have to grade excellent for every unit of the score. In our present system this would not be necessary. For instance: should a cake grade excellent in all but color and the color grade below five, which would be considered poor, the final score on the cake would be noted as poor on account of color. The same rule would apply to any of the characteristics of the cake, such as symmetry, texture, or grain.

Table III gives the analysis and the description of the flours used in this year's work.

TABLE III

ANALYSIS ON FLOURS[1]

1939 CAKE COMMITTEE FLOURS

A—Commercial cake flour.
B—Commercial cake flour.
C—Commercial pastry flour, chlorine bleach.
D—Commercial all-purpose family flour, Agene bleach.

					Viscosity		
Sample	Protein	Ash	Moisture	pH 30°	20 g. flour	2 g. protein	No time
	%	%	%		°MacM.	°MacM.	°MacM.
"A"	7.5	.348	11.9	5.24	54	108	41
"B"	7.4	.368	12.0	5.08	47	111	34
"C"	7.4	.415	11.7	5.40	44	97	29
"D"	10.3	.402	12.3	5.80	136	124	109

[1] Prepared by V. H. Morris, Ohio Agricultural Experiment Station.

Cakes were baked using the present A.A.C.C. formula and a modified A.A.C.C. formula set up with 90 cc. of egg white and 90 g. of

shortening.   Both layer and loaf cakes were baked from each batter. Cakes were judged according to the present A.A.C.C. score system and the proposed score system (Table II), use being made of Stamberg's photographs for grain and symmetry judging in this work.[1]

Character of the flours was varied over a large enough range so that grain and texture differed considerably.   Using either loaf or layer cakes and reporting by either scoring system, all members of the committee classified the cakes in about the same order, there being some difficulty in deciding on flours A and B between collaborators.

Summary

Committee members disagreed on the value of layer and loaf cakes for testing.

No agreement was reached as to whether volume should be reported in the score or merely by number of cubic centimeters.

Few members favor the use of flavor as part of the score card.

Members all agreed on value of "stop" system in scoring.

Recommendations for Future Committees

Immediate steps should be taken to provide descriptive terms for present score card.

Further study should be made on a "stop" system for the present score card.   .

Both layer and loaf cakes should be considered.

Measurement should be made on pH of the finished cake.

In scoring grain: (a) an attempt should be made to further complete the Stamberg photographs, and (b) cake slices should be prepared for scoring.

The break on top of a loaf cake should be considered when scoring symmetry.

Further attempts should be made to standardize on equipment between collaborators.

[1] Cereal Chemistry 16: 764.

REPORT OF THE 1939–40 COMMITTEE ON THE STANDARDIZATION OF LABORATORY BAKING

QUICK LANDIS, *Chairman*

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The impending revision of the Book of Methods has forced the committee to a somewhat hurried consideration of the whole aspect of the baking test. Steady progress has been made since the last revision and many of the improved features are sufficiently well established to permit their incorporation into the method.

In view of the difficulties which have arisen with respect to the availability of a single mixer, particular emphasis has been placed on calibration. To facilitate calibration it would be desirable to have an "analyzed" sample of flour continuously available to our members. The Association might wish to consider this possibility.

Satisfactory methods for reporting and interpreting results are not yet well developed. Characterization of loaf types and internal characteristics as an aid to this problem should be included in the Book of Methods. The committee will undertake this project and hopes to have it ready in time to incorporate in the revision.

Although the pan situation is still unsatisfactory the committee has as yet insufficiently complete data to warrant recommendation of change in the pan sizes previously specified.

The committee has concluded that the rolling pin method of hand manipulation is definitely superior to that previously described, and thus recommends the substitution of the improved method when machine method molding is not available.

Accordingly it is recommended:

1. That Cereal Laboratory Methods be amended according to the recommendations submitted.

2. That the Association consider the appointment and maintenance of a committee to prepare, analyze, and periodically refresh a sample of flour to serve for mixer calibration.

3. That the succeeding baking test committee continue its efforts toward the development of the baking test with particular emphasis on reporting of results.

BOOK REVIEW

Official and Tentative Methods of Analysis. Association of Official Agricultural Chemists, Washington, D. C., 1940. 757 pages.

This new edition continues the tradition of the Association of Official Agricultural Chemists of selecting the most reliable methods of chemical and physical analysis used in regulatory and research work.

The book shows a marked expansion in the work of the Association. In the Fourth Edition, six chapters were given without text. The chapters on Fish and Other Marine Products, Vitamins, Microbiological Methods, and Microchemical Methods have been developed in this edition, leaving only the chapters on Sewage and Agricultural Dust without text.

The increased activity of the Association has also resulted in numerous additions to the individual chapters. For example, the chapter on Cereal Foods has increased from 79 subdivisions in the Fourth Edition to 99 in the Fifth Edition. Methods for sugars (reducing and nonreducing) have been added to this chapter.

A different method for the determination of chlorine in fat is given. The increased use of soya beans has developed a need for the detection of soya flour, and a qualitative test is given for soya flour in uncooked cereal products. Similarly, the chapter on Grain and Stock Feeds has increased from 56 to 68 subdivisions. A method for determining fat acidity in grain has been added. A method is presented for the determination of manganese, the element needed in the diet of poultry to prevent perosis, or slipped tendon. The increased interest in vitamin A activity has resulted in the addition of a method for the determination of carotene in hay. After the extraction and separation of the carotene, three choices for estimation are given. Either a spectrophotometer, photoelectric colorimeter, or visual colorimeter can be used. A table is given for using potassium chromate solutions for standards of comparison for the latter two types of measurements.

Additional methods of analysis in the chapter on Dairy Products has resulted in an increase in the number of subdivisions from 110 to 135. The methods of analysis that have been added to this chapter include: the determination of citric acid in dried milk, a test for mold mycelia in butter, a method for gums in soft curd cheese, a phosphatase test for pasteurization, and bio-assay for vitamin D in milk.

The reviewer would not undertake to report all the changes in the three chapters cited. The examples were chosen merely to show the numerous additions and changes that the Association has made in this new edition. Similar additions and changes can be noted throughout the book.

The revision of subject material has necessitated an increase over the Fourth Edition of only 47 pages. This small increase in the size of the book has to some extent been made possible by the use of the term *ca* for *approximately* and *about*, the partial elimination of the articles *a*, *an* and *the*, and other methods of presentation employed by the editors to save space without the loss of clarity.

The book is worthy of replacing its predecessor on the bench of the chemist who is called upon to make analyses of farm products and related materials.

<div align="right">Kenneth T. Williams</div>

CEREAL CHEMISTRY

GAS PRODUCTION IN YEAST FERMENTATION AND ITS APPLICATIONS. III. THE BAKING TEST

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This test has very likely been the subject of more discussion than any other test or group of tests employed by cereal chemists in the study of panary fermentation. It is therefore with hesitation that one attempts to add a word to an already lengthy chapter on a procedure which by its very empirical nature changes with the opinion of the individual workers.

We may state at the outset that the baking test, and by the baking test we mean the entire procedure from mixing of dough to staling of loaf, has proved an invaluable guide to us in at least five distinct directions: (1) blending of wheat, malt flour, and bleaching agents of the maturing type in the maintenance of flour mill specifications, (2) selection of flours best suited to the needs of individual bakeries and maintenance of uniformity in such selections, (3) study of supplementary ingredients, particularly milk and other improvers generally referred to as *dough conditioners* or *yeast foods*, (4) study of yeast, and (5) both pure and applied research. The test undoubtedly proves informative in other fields such as in the selection of new wheat varieties. These, then, constitute our reasons for employing the baking test and insofar as the test fulfills these expectations it will be useful. It is to be noted that the first of our applications of the baking test coincides with the first of its two purposes as seen by Fisher and Halton (1937). Our fifth application includes but goes far beyond their second purpose in that we do not necessarily observe dough behavior with the concept of flour utility in mind; rather, we are interested in the mechanism of that behavior. We wish to know not whether a flour is a short or a long fermentation-time flour, but why it is so and how we can make it otherwise. The second of our appli-

cations is similar to, though not identical with, that of the second of the two stated by Davis (1937). He employs the test as a means of finding the best baking procedure for a given flour, whereas we attempt to find the best flour for a given baking procedure. Landis and Frey (1936) also claim two fields of usefulness for the baking test, their first coinciding with the first of Davis (1937). They employ it as a means of evaluating characteristics which can be determined in no other way. This we take to be self-evident, except that the question as to which characteristics fall into this class remains unanswered. Their second is that the baking test serves as an overall check upon factors determined by other methods. It occurs to us that these other methods must be poor indeed if the baking test must serve as a check upon them, in view of the statement of Davis (1939) that collaborative studies of the baking test were so discouraging as to make it inadvisable to continue the collaborative work. In reference to this, one may conclude with Blish (1936) that standardization at least of the mechanics of the baking test must and will in due course of time precede successful experimental agreement among individual laboratories.

Type of Procedure

Much has been said in recent years concerning the mode of attack to be used in designing a baking test. Davis (1937) speaks of two schools of thought, that which adheres to a *fixed* and that which adheres to a *variable* type of procedure. Landis and Frey (1936) distinguish between *fixed ingredients* and *fixed factors*. They indicate that either the ingredients or the factors but not both may be fixed. With this premise we are in complete agreement; with the deduced consequences, however, we find a tendency toward reaction to a now in a sense obsolete school of thought, namely, that one which strives to approach *optimum conditions*. In this school we may place those who attempt to duplicate commercial bakery practice, since the baker ever strives for optimum conditions. Hence, the *optimum condition* school survives as the true philosophical opponent of the *fixed condition* school, which itself is now divided into two subgroups of *fixed ingredients* and *fixed factors*. That *fixed factors* are far more to be desired than *fixed ingredients* has been excellently demonstrated by Landis and Frey (1936) as well as by many others. So much then is clear, but it is also true that the subtle dangers inherent in the technique of fixed factors have not received their warranted publicity. To this we will return after first discussing the apparent position of those who argue for *optimum conditions*.

Optimum vs. Fixed Conditions

Proponents of the *optimum condition* school hold that the experimental conditions should be so varied as to permit a flour in question to display its utmost virtues; that is, the optimum conditions of all controllable variables must be found before practical interpretations concerning flour utility can be drawn. It is at once apparent that this technique by its very nature is subject to scientific treatment only under two distinct sets of conditions. The first demands that individual tests be performed for each complete set of permuted variables. This, the so-called *Latin-square technique,* demands practically unlimited materials and unlimited time, and hence is of little practical significance. It may be argued that the judgment of the individual experimenter is such that many of the variables legitimately may be neglected because of automatic intuitive compensation for their various interactions. That is, it is unnecessary to perform the baking test for that group of permuted variables in which the fermentation period, for example, is varied by definite increments of time, because the intuition of the experimenter will more likely than not indicate the proper and optimum fermentation time.

There may be some who will question the use of the word *intuition.* They will say that the experimenter judges the time necessary for optimum fermentation after due consideration of the observable characteristics of the fermenting dough. The mental road, nonetheless, remains intuitive in nature as long as the experimenter does not understand at least the pertinent causes and mechanisms involved in the production of the observed characteristics.

That this is so, is an experimentally demonstrable fact. It is not uncommon to find independent fermentation experts in earnest disagreement as to the *youth, optimum,* or *age* of a fermenting dough. Their disagreement often becomes more marked upon comparison of their opinions of the finished loaf. Some loaves are said to have *old* characteristics, some *young,* and some are *young* in certain respects while *old* in others. This has been borne out beautifully on several occasions by the following technique. Two series of six loaves each were prepared by the sponge-dough procedure shortly to be described, the first series from 3½-hour sponges and the second from 12-hour sponges. The resulting loaves were presented to two men thoroughly familiar with the practical aspects of dough fermentation. In this instance, they both agreed that the loaves produced from the 3½-hour sponges possessed symptoms of *age,* while those produced from the 12-hour sponges possessed symptoms of *youth.* When their error in judgment was pointed out, they parried by asserting that the loaves produced from the 12-hour sponges were so *old* they had reclaimed the

symptoms of excessive *youth*.   One finds no argument with this pos-
sible caprice of nature, but does find scientific unreliability in the
technique that permits ambiguous and ill-defined judgments.

This process of reasoning permits us to arrive at two conclusions.
First, it is humanly impractical to study all the permuted variables
in the attempt to find the optimum conditions for a given set of raw
materials.   In fact, it is humanly unattainable, since an infinite
number of variables is involved.   Second, the problem may not be
simplified with legitimacy by trusting to the uncertainties of the
experimenter's judgment.   To do so transports the study from the
realm of science to that of art.

We may now turn to the second set of conditions necessary to
permit the study of flour or supplementary ingredients under optimum
states of all concerned variables.   This demands that we first study
complete sets of permuted variables sufficient in number to permit us
to arrive at the general laws involved; that is, before any routine study
of flour or supplementary ingredients is approached, we attempt to
develop some understanding of the fundamental causes and mecha-
nisms involved in the production of observable characteristics.   Once
these laws are evaluated, it will no longer be necessary to study each
of the infinite groups of variables, but some selected few will suffice to
permit us by deductive reasoning to determine the optimum conditions
of variables for the particular ingredient under consideration.

Hence to apply scientifically the method of the school demanding
optimum conditions, we must first obtain some analytical understand-
ing of the physical, chemical, and biological aspects of fermentation.
This it must be agreed we do not as yet possess.   Hence we are led to
conclude that necessary preliminary work must precede the applica-
tion of this technique to the scientific rather than the artistic study of
flour as well as to the study of supplementary ingredients.   But we
wish to continue with the concept of optimum conditions, the pathway
is clear.   We must first acquire the necessary understanding of the
processes of fermentation and this may be attempted by working under
fixed conditions, permitting only one variable to change at a time.
We may thus study the effect of the given variable and make the
opportunity for discovering the laws connecting the observable char-
acteristics with each of the variables concerned.

It is to be hoped in fact that appropriately directed study will lead
to the solution of a significantly large number of such relationships
and ultimately to a solution of the complete relationship.   But this is
just the procedure advocated by the second of the two schools of cereal
chemists.   It would seem, then, that the continued existence of these
two schools of thought is not particularly conducive to the rapid

development of the science of cereal chemistry. This difficulty may well be illustrated by a study of much of the published data. These cover a multitude of conditions and a multitude of interests. Relatively few, however, represent an exhaustive study of some single effect. Hence if one desires to generalize and ascertain even elementary principles, he is at a loss through lack of correlative data.

The Standard A.A.C.C. Baking Test was originally designed with concepts similar to these in mind. Unfortunately, the *optimum condition* school has had its influence even here. For example, the Tentative Baking Test as published in the third edition of *Methods of Analysis* of the A.O.A.C. in 1930 requires that absorption be kept constant, while the 1935 edition of *Cereal Laboratory Methods* suggests that absorption be varied to suit the flour; that is, the resulting dough must be neither too slack nor too stiff. No criterion for judging this will-o-the-wisp, however, is indicated. Variation of absorption to suit the flour is taken to mean optimum absorption.[1] But whether by this is meant that absorption which produces the optimum grain or optimum volume, or optimum loaf (itself as yet not quite defined) or yet perhaps optimum mixing characteristics, is left to one's intuition. But it is just this procedure which has been shown to be unsound. It might prove well worth while to reread the article entitled "The Baking Test," by E. E. Werner (1925).

One final thought in regard to this matter is perhaps suggestive. It may be granted that over a period of years a selected group of individuals will gather sufficient experience to warrant respect for their judgments concerning optimum fermentation time, temperature, absorption and the like; and their interpretations of flour and supplementary ingredient characteristics will prove as sound as their judgments. Nonetheless, their results remain irreproducible by fellow workers, and at the same time relatively useless as data upon which theoretical study of general principles can be based. It would seem that this same selected group of individuals, rather than sharpening their senses in the direction of nonuniversal, artistic irreproducibles, might better have spent their years developing similar sharpness of intuition in the direction of interpretation of results obtained under fixed, universal, reproducible conditions. *A priori*, their chances for success by route of this second procedure are at least as great as their chances for success by the first procedure, and at the same time their compiled data could serve as a foundation for fruitful penetration into the sundry mechanisms of fermentation.[2]

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[1] See Markley and Bailey (1938) for a discussion of this point.

[2] The preceding discussion may very likely have some bearing on an often repeated criticism of the basic test. To quote Markley (1940): "The basic test, as devised by Dr. Werner, was a test of diastatic activity or gassing power of a flour rather than the strength." It occurs to us that Dr. Werner

We may now decide that whether our interest lies with flour characteristics or with supplementary ingredients, our choice of procedure must be restricted to those that permit working under fixed, reproducible and certainly standardized conditions in which only that one of the variables which is under immediate scrutiny is permitted to change. Different workers in different localities studying the same effects must obtain at least correlative if not identical data. Those factors which disturb this possibility should be studied one by one so that adequate corrections for uncontrollables like barometric pressure and yeast variations can be given due consideration in interpretation of data.

Fixed Factors vs. Fixed Ingredients

Having already conceded the validity of the arguments of Landis and Frey, we return to the dangers inherent in the method of *fixed factors*, and to the necessity of avoiding the road towards *optimum conditions*. We perceive two major pitfalls.

The first is elementary, though nonetheless overlooked. We take the case of determining the potential sugar level of flours prior to their study via the baking test. These authors indicate an empirical method for determining the potential sugar level and advocate varying the ingredient sugar among the flours so that all will have a common potential sugar level. They give directions for determining either the amount of sucrose or of nondiastatic malt to be added. But Larmour and Bergsteinsson (1936) have shown that the rate of gas production is affected differently by sucrose than it is by maltose. Further, Sandstedt and Blish (1939) restate their earlier findings that a common sugar level does not guarantee a common rate of gas production. Larmour and Brockington (1934) also have shown there is a response due to sugar independent of its effect upon gas production. By controlling only the potential sugar level, therefore, we cannot control the rate of gas production. Further, there is no reason to believe that controlling the potential sugar level without due consideration of the kinds of sugar will eliminate the specific effect of Larmour and Brockington.

The work of Kuhlmann and Golossowa (1936) on bound water as affected by maltose may be of interest in this respect. In addition,

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himself very likely was familiar with other more satisfactory physical and chemical methods for the determination of diastatic activity or gassing power and hence would not have used the relatively cumbersome baking test for this purpose. It further occurs to us that, realizing the hopelessness of the *Latin-square technique*, he may have tentatively selected the basic test as one distinguished by the highest return in information for the amount of labor involved; that is, diastatic activity was learned and this information used in the interpretation of volume and so on. Data obtained by the quantitatively fixed procedure of the original basic baking test are but a step from possessing absolute significance, in contradiction to the opinion expressed by Markley. The whole argument hinges upon the position of judgment. Shall we employ judgment after the data, in a fixed and universally reproducible manner, are acquired? Or shall we employ judgment before the data are acquired?

this laboratory has found the rate of gas production particularly in straight doughs to be somewhat sensitive to variations in sucrose. This effect as will be shown in a subsequent communication is often negative during certain periods of fermentation and very likely explains the negative volume response to sucrose found by Sandstedt and Blish (1939) for flour number six. That is, with a wheaten dough as substrate, maltose fermentation is inhibited by sucrose. This further explains the observation of Bohn and Favor (1939), "that the rate of gas production is faster during the third hour when no sugar is present than at any other time." The work of Schultz and Atkin (1939), however, in which dextrose is found to *stimulate* maltose fermentation cannot be considered in disagreement with our findings, since their buffered liquid substrate presents an entirely different environment for the fermentation.

We may conclude, therefore, that though it is desirable to have all flours at a common sugar level, the method of Landis and Frey does not serve satisfactorily; the problem is far more complex than indicated by them. Certainly a common rate of gas production during the proofing period is more to be desired than a common empirical potential sugar level. The first pitfall, then, lies in too quickly applying a means for controlling a factor at the expense of an ingredient when sufficient knowledge concerning that factor is not at hand. The method suggested by Sandstedt and Blish (1939) of supplying an excess of sucrose and proofing to constant height very likely is more satisfactory, though we may here indicate that we have reason to believe that employing an excess of maltose rather than sucrose with the simultaneous addition of about 0.07% ammonium chloride will bring most flours to a common and relatively constant rate of gas production during the third hour of fermentation, all other factors abiding by the A.A.C.C. procedure.

We may now turn to the second and more subtle pitfall that must be avoided in attempting to fix factors rather than ingredients. Varying absorption to suit the flour has already been mentioned. Landis and Frey (1936) indicate that flours may vary from 57% to 75% in this respect. They do not mention, however, that because of the unfortunate manner in which cereal chemists compute percentages, the actual yeast content, sugar content, salt content, and protein content of the dough are forced to vary as the absorption is varied. We compute that yeast will vary from 1.875% of the dough weight in the low absorption flour to 1.685% of the dough weight in the high absorption flour; that is, the former will contain 11.3% more yeast than the latter. The question is one of selecting the factor to be fixed. Certainly constant water content of the dough is desirable, just as con-

stant potential sugar level may be. Shall we select constant water content or constant dough consistency as the factor of interest? The former can be determined with relatively universal accuracy; the latter is a function of rather complex and certainly arbitrary instruments—when these instruments are available.

The matter of *fixed factors*, if appropriate caution is not exercised, may further take one into the realms of the near-absurd. Shall we not correct all flours to a common protein content, either by adding gluten or by adding starch? We do not wish to cast reflections on the recent excellent work of Sandstedt, Jolitz, and Blish (1939) in which they report separating the starch from the gluten and subjecting these individually to study. Theirs is certainly a sound attack just as the one raised by our question above is unsound. The second pitfall to be avoided in using fixed factors, then, lies in fixing one factor at the expense of another possibly more important one.

The Role of Gas Production

As pointed out by many writers the conventional baking test produces a loaf the characteristics of which are a function of at least two distinct groups of effects; in the first we may place the rate of gas production and in the second all factors other than the rate of gas production. It is apparent that a simple method for separating the rate of gas production as a variable would then permit a far simpler interpretation of the baking test. This, too, has been the subject of much discussion. Concerning the techniques for accomplishing that separation, however, there is much disagreement. Perhaps the one most employed at the present time consists of proofing to constant height rather than to constant time.

We will first attempt to give analytic expression to the theoretical weakness of this technique, and then will consider the selection of a method which has indications of being superior. As a starting point we may estimate the volume V' of the molded dough after pan proof and immediately prior to entrance in the oven. It is equal to the sum of the volume of the dough at molding, A, and that proportion of the gas produced during proof which is retained.

Hence

$$V' = A + \int_{t_1}^{t_2} P_{ex} \left(\frac{R_P - R_L}{P_i} \right)_t dt, \tag{1}$$

where t_1 represents the time of molding, t_2 the time at which pan proof is ended, R_P the rate of gas production, and R_L the rate of leakage at time, t. P_{ex} signifies the external atmospheric pressure and P_i the average pressure within the dough at this time.

Both R_L and P_i may be considered complex functions of the size and shape of the dough mass as well as of R_P and V'. The ratio P_{ex}/P_i may be neglected only insofar as the dough is made sufficiently small, for under this condition the relative dough surface increases, the total rate of leakage approaches R_P and as a consequence P_i approaches P_{ex}. We may tentatively assume, therefore, that the elimination of P_{ex}/P_i for 8.00-g. doughs is justified. The fact that gas-production data acquired from this size of aliquot show no response to those quantities of potassium bromate which are known to affect the physical properties of the dough may be considered as evidence. Such gas-production measurements may then be considered to determine R_P rather than $P_{ex}/P_i R_P$, particularly if such measurements are to be employed in interpretation of a baking test performed on a relatively larger dough. The rate of expansion of the dough [3] at time t is therefore represented by $R_P - R_L$ for small doughs and by $P_{ex}/P_i(R_P - R_L)$ for large doughs.

Rather than thinking in terms of size it is perhaps better to think in terms of relative free surface. It follows that if one wishes to remove R_P as a variable affecting the baking test, then neither proofing for a constant period of time nor proofing to a constant height is a suitable attack. For if one assumes that variations in A with variations of flour or variations of supplementary ingredients are negligible compared to the variations in the integral of equation No. 1, then the former method merely limits the range of the integral while permitting R_P and R_L and the integral to vary. The latter method removes the time limitations upon the integral in a fashion such that the integral always has a constant value, permitting nonetheless both R_P and R_L wide fields of variation.

This would at first sight seem unimportant. For here at least all doughs upon entering the oven apparently contain similar if not identical volumes of gas. It would seem then that the loaf volume would measure some average rate of leakage. But unfortunately this may be far from the truth, since at least during the first three or four minutes of baking (A.A.C.C. procedure) the dough undergoes expan-

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[3] It is of interest to note that many workers, as for example Elion (1939), call the maximum value of the integral

$$\int_{t_1}^{t_2} P_{ex} \left(\frac{R_P - R_L}{P_i} \right)_t dt$$

the *gas-retaining capacity* or *retention*. It is not usually mentioned, however, that this characteristic is a function of gas production whether the rate of gas production is small or large, as well as it is of the rate of leakage. It is important that whereas R_P has clear-cut physical significance, R_L is itself dependent upon V' and hence upon R_P and is therefore of rather obscure physical significance. In fact, it may be concluded that the *gas-retaining capacity* can be of little value as a flour characteristic unless the behavior of R_P as a function of time (a factor in the sense of Landis and Frey) can be made common to all flours studied at the expense perhaps of varying an otherwise relatively unimportant ingredient or condition.

sion and both R_P and R_L during this interval may behave quite erratically. For example it has been our experience that many flours which possess large positive bromate responses often proof lower in the pan in the plus rather than in the minus dough; that is, the bromate response is demonstrated by oven expansion rather than by expansion in the pan. Since it can be shown that potassium bromate in the quantities normally used (0.001% based on flour) does not affect gas production, it would seem that the average rate of leakage, $\bar{R}_L$, during pan proof is greater for the plus dough, while the average rate of leakage, $\bar{R}_L$, during baking is less for the plus dough.

On the other hand, it may be that the plus dough has been so changed that greater force is required to produce a given amount of pan expansion, and that rather than $\bar{R}_L$ being greater, it is actually smaller, and the apparent lack of pan expansion is due to compression of the gas within the dough structure. Whatever the explanation, it remains that neither technique, fixed time, nor constant height of pan proof really separates gas production from gas leakage or from the baking test. Ideally, it remains to design a baking test which automatically maintains a common rate of gas production during pan proof and during the first five minutes, say of baking, independent of the supplementary ingredients. If this cannot be done then at least criteria may be attempted whereby appropriate legitimate corrections may be applied. The latter in general is the simpler attack.

Basic vs. Primitive vs. Commercial Formulas

Before proceeding it may be wise to define certain terms: *Basic ingredients* has been taken to mean flour, water, yeast, sugar, and salt. *Commercial ingredients* for the present purpose refers to flour, water, yeast, sugar, salt, milk, and shortening. The use of the description *primitive ingredients* refers to flour, water, and yeast. Any ingredient not included in the above definitions is classed as a *supplementary ingredient.*

In the study of flour, American cereal chemists have adopted the basic formula as one in which flour plays the prime part and which therefore permits the flour to display its characteristics under laboratory conditions. The problems involved in the study of supplementary ingredients, however, are different at least in scope. The supplementary ingredients may interact not only with the flour but as likely as not will interact with any or all of the commercial ingredients as well. Hence a commercial rather than a basic formula is indicated for work of this type. In addition, some supplementary ingredients, particularly insofar as gas production is concerned, may initiate different responses dependent upon whether the fermentation is principally

one of maltose or one of sucrose. This leads to the selection of a primitive formula as well as a commercial one; that is, we may be interested in how the supplementary ingredient affects gas production in the sponge stage (primitive formula), how it affects gas production in the dough stage (commercial or basic formula), and finally how, after consideration of effects upon gas production during proof and early baking, it affects the finished loaf.

Straight Dough vs. Sponge-Dough Process

If it is granted that supplementary ingredients may affect properties other than gas production, and may produce greater or less or even opposite effects depending upon their time of contact with the fermenting dough and if it is further granted that in the study of flour, characteristics other than those of gas production also vary with the age of the dough, then it follows that the sponge-dough procedure, far more readily than the straight dough, permits one to determine such effects.[4]

The work of Frey, Freilich, and Ekstedt (1937) on sponge doughs forms our basis for this statement. We quote: "It may therefore be concluded that after a minimum sponge fermentation of about $3\frac{1}{2}$ to $4\frac{1}{2}$ hours, depending on the temperature of the medium within which they are set, and using the type of flour and formula given above, laboratory sponges may be fermented within a wide range of time— up to 7 hours, and possibly more, and then produce bread of good quality." Our experimental findings will serve to amplify their observations and will verify the *a priori* judgment that this stability towards changes in sponge time is to be expected except under more or less unusual circumstances.

This tolerance will be shown to rest upon the fact that gas production during the dough stage of a properly conducted sponge-dough procedure is relatively independent of sponge time for a given flour after some minimum sponge time depending upon a predeterminable characteristic of that flour. In fact, it is evident even at this point that the matter of potential sugar levels is naturally solved by the sponge-dough technique—particularly if the sponge percentage is kept high relative to the dough percentage. The problem of common level

[4] That Shellenberger and Ziemke (1939) found the sponge-dough procedure less informative than the straight dough we cannot accept as a valid objection to the former technique. In the first place their formula included those ingredients "used by the average baker." Several objections to this procedure other than those already raised are evident: (1) In a test already suffering through lack of a standardized ingredient (yeast), other even less standardized ingredients (milk, shortening, and malt) are superimposed. (2) Yeast food, at times not sufficiently homogeneous in composition to escape notice by the commercial baker when used in relatively large quantities (a pound or more) is here used in an analytical test in the relatively small quantity of a gram or less. (3) Milk itself has a stabilizing effect upon at least certain flour responses as pointed out by Ofelt and Larmour (1940) and Eisenberg (1940). In the second place they employ a standard sponge or dough flour when testing dough and sponge flours respectively. Determining whether a flour is suited for sponge or dough use itself is one of the many objects of the baking test as we understand it, and hence the flour under consideration must not be masked by blending—certainly not in any method which is to serve as *basic*.

of gas-production rates for all flours, however, remains unsolved as is to be expected since important variables other than sugar level as yet are uncontrolled. Possibly thiamin (Schultz, Atkin, and Frey, 1937) and certainly amino nitrogen play their respective parts.

Experimental

This work as indicated in the title concerns itself particularly with the study of gas production, though in the present instance application of such data to the development of a baking test is attempted. Two formulas are selected as points of departure, one for the study of supplementary ingredients, another for the study of flour. For the latter purpose, in this preliminary probe, we employ the *basic* formula of the A.A.C.C.; [5] for the former we accept a commercial formula in which 4% of powdered skim milk (spray), 4% of hydrogenated cottonseed oil, 2.5% sucrose, and 1.0% sodium chloride (all based upon flour weight) are superimposed upon the *basic* formula. Absorption is kept constant at 60% [6] (15% moisture basis) for both sponge and dough, though tentatively 1% additional water is used for each percent of milk powder. Supplementary ingredients unless otherwise mentioned are incorporated in the sponge. Three percent of yeast *A* based upon sponge flour is used throughout. Thus an aliquot may be taken for the determination of the rate of gas production (primitive) in conformity with the procedure established in Part II of this series.

One modification of this procedure in which the preparation of the sponges is described in detail may be noted. Only one sponge is to be mixed each quarter hour, instead of two, but this in sufficient quantity to prepare a dough large enough for two 150.0-g. and two 8.00-g. aliquots. The former are to be employed in the baking test, the latter in the determination of the rate of gas production (dough stage) as a function of time.

The sponges are fermented in a thermostatically controlled proof box at 30.0 ± 0.1° C. for lengths of time as indicated in the experimental data. Doughs are mixed in a single-speed Bachmann mixer as follows: All soluble ingredients are dissolved in the prescribed amount of water. The flour and shortening are placed in the mixing bowl, whereupon the milk is taken up in the water and also added. The sponge is then superimposed and mixing proceeds for one minute.

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[5] The acceptance of this formula by ourselves has been only tentative. Since the data of this paper have been acquired, we have been investigating a new formula which may be identified as the *reference formula*. This applies particularly to sponge-dough use and consists of 3% yeast based upon sponge flour (80% sponge), and 5% sucrose, 2% sodium chloride, and 60% absorption based upon total flour. This change is based upon two circumstances. First, the sugar and salt contents are hereby brought into correspondence with those of the commercial formula. Second, a sharply declining rate of gas production versus time curve in the dough stage is avoided.

[6] For those who may object to this technique it may be noted that either increases or decreases in absorption of at least 4% do not affect rate of gas production data tangibly. This is in agreement with the conclusion of Halton (1938) and with Heald (1932).

Twenty seconds are now taken for cleaning the bowl with the aid of a large spatula. Alternate mixing and cleaning are then continued in 40-second and 20-second steps, respectively, until the dough has received a total of three minutes of mixing. Aliquots for gas-production study are taken immediately and the remaining dough is permitted to rest for 25 minutes. It is then scaled and molded by means of a Model G Thompson Roll Molder. The molded dough is placed in the high-type aluminum pan and proofed for 55 minutes at 30.0°C. Baking is done at 230 ± 3°C. in a thermostatically controlled Despatch oven equipped with a rotating platform. Loaf volumes are measured to the nearest 5 ml. by displacement of rape seed.

Gas-Production Data

Since we have recently decided in favor of 80% sponges, and since all experimental work to be done by us in the future will be restricted to this condition, it is perhaps unfortunate that certain of the data immediately to be discussed were obtained via 50% sponges. But we
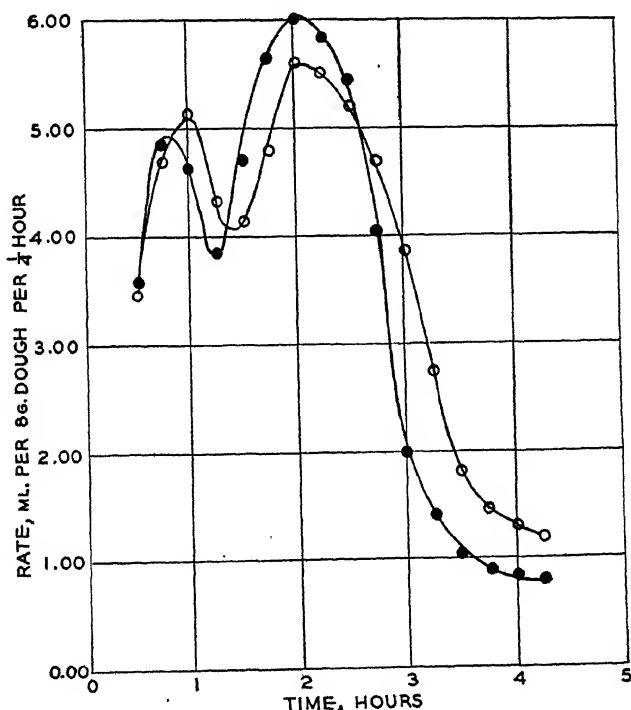
Fig. 1. Sponge curves for flour LS9c and flour LS11. The former is represented by closed circles and the latter by open circles.
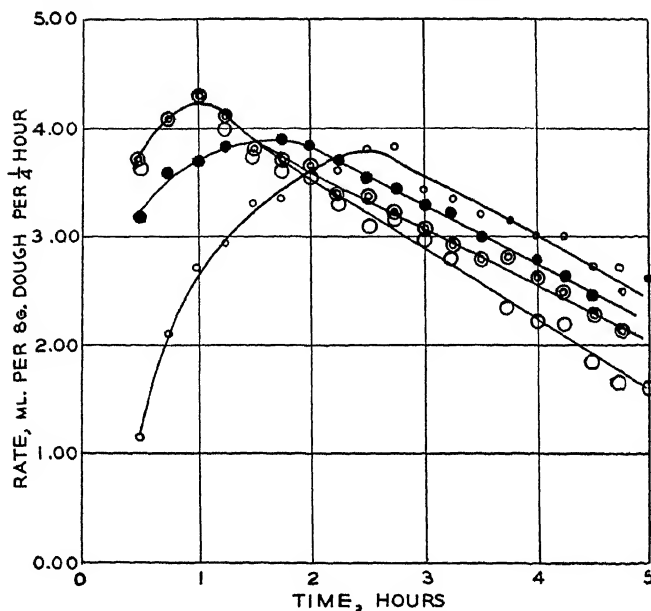
Fig. 2.   Dough curves via basic formula, 50% sponge and flour LS9c for various sponge times. Small circles, no-time sponge; closed circles, 1 hour; double circles, 2 hours; and large circles, 3 hours of sponge time.
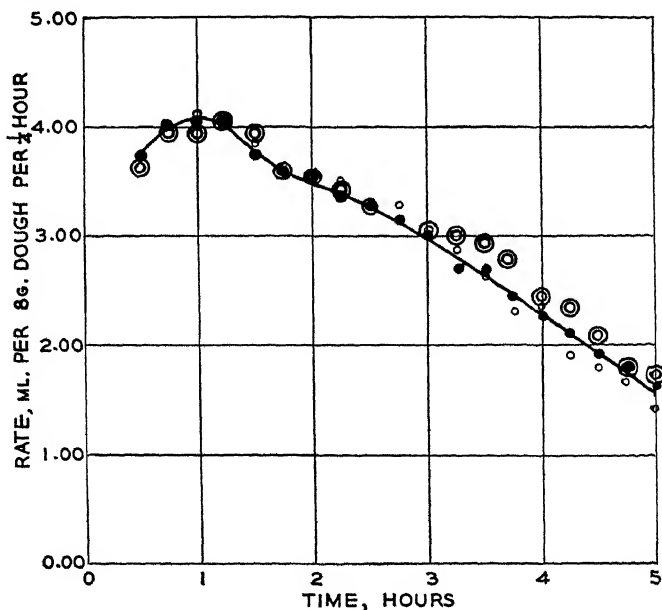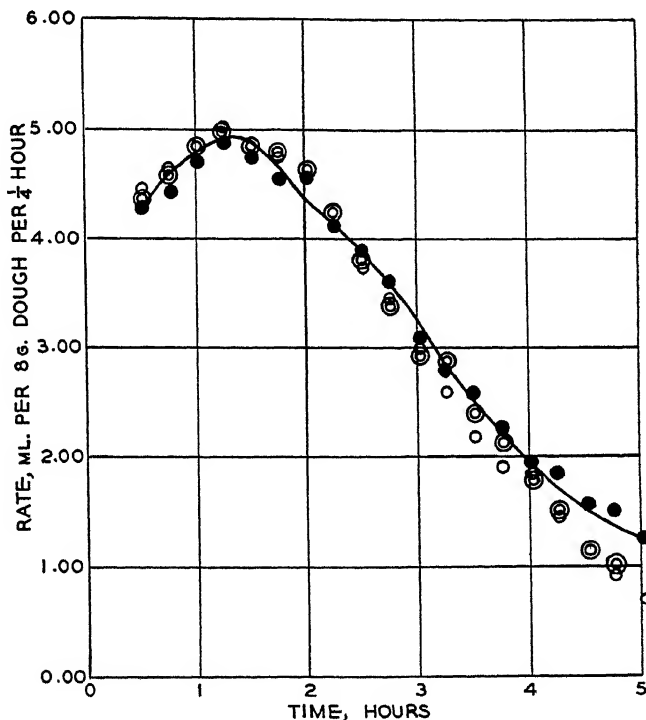

Fig. 3.   Dough curves via basic formula, 50% sponge and flour LS9c for various sponge times.   Closed circles, 3½ hours; open circles, 5 hours; and double circles, 5½ hours of sponge time.

think their presentation at this time is justified nevertheless as an indication of the generality of the principle to be drawn, namely, that the rate of gas production in the dough stage for any given set of ingredients and conditions is, after some minimum sponge time, relatively independent of the sponge time.   All data other than those given in conjunction with simultaneously obtained baking data are corrected to standard pressure and temperature; those given in conjunction with the baking data are corrected only to standard temperature.

Basic formula—50% sponge—flour LS9c.—This flour, one intended for baker's sponge use, possesses 11.9% protein and 0.40% ash, and is here used both in sponge and in dough.   Figure 1 represents the rate of gas production as a function of time during sponge fermentation.[7]   The leveling off of the rate of gas production to a relatively linear decline of small slope after some 3½ hours is evident.   This is in agreement with the findings of Sandstedt, Blish, Mecham, and Bode (1937).   As pointed out by them, this very likely indicates complete consumption both of native and readily formed sugars.   The persistence of gas production after this point is very likely due to the slow formation of additional sugars from nonsusceptible starch by an alpha-amylase-like enzyme.   If this is true, it follows that all sponges taken after this time must possess a practically common sugar level.   Hence all doughs mixed from sponges of 3½ hours or greater age with the flour under consideration should yield common rates of gas production vs. time curves.[8]   This is experimentally established in Figures 2 and 3.

Basic formula 50% sponge—flour LS9c—0.05% NH₄Cl.—The group of dough curves obtained under these conditions and illustrated in Figures 4 and 5 serve as supporting evidence for our thesis.   One interesting side issue is worthy of note.   The work of Larmour and Bergsteinsson (1936) has already shown that there is no rate of gas-production response to ammonium chloride during the first 2 hours of fermentation under their conditions.   We may qualify our agreements with their findings by a matter of some 30 minutes; that is, we have found ammonium chloride responses in straight dough procedures at about 1½ hours and longer.   In the present instance one may note by comparing these figures with Figures 2 and 3, that the time at which the ammonium chloride response becomes evident is an inverse function of the sponge time, decreasing to zero at a sponge time of some 2½–3 hours.

Another pertinent point rests with the fact that the ammonium chloride peak seldom precedes the control peak.   In fact, it usually

[7] Curves of this type will hereafter be referred to simply as *sponge curves.*
[8] Curves of this type will hereafter be referred to simply as *dough curves.*

follows it.   This has been verified under various other conditions and with many other flours.

We may combine these two observations with a third, namely, that since more gas has been produced prior to the peaks in the presence of ammonium chloride than in its absence and since it can and will be shown at another time that the ammonium chloride effect is not tangibly associated with diastatic activity, contrary to the surmise of Larmour and Bergsteinsson (1936), there is less sugar remaining in the
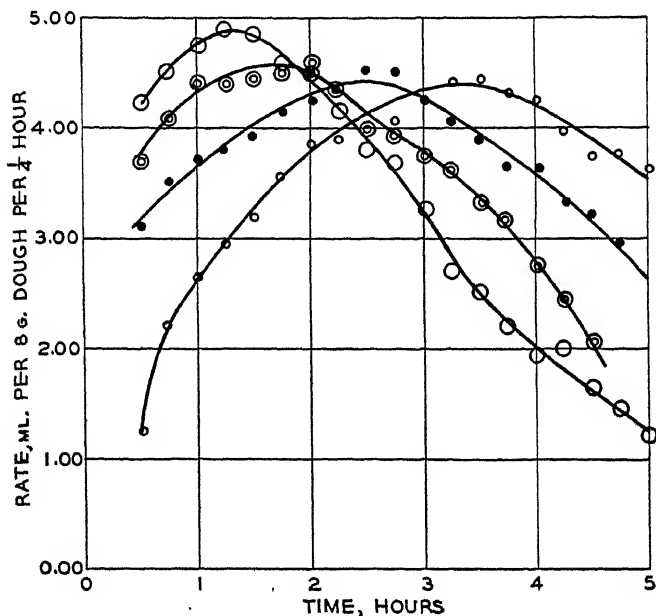
Fig. 4.   Dough curves via basic formula, 50% sponge, 0.05% ammonium.chloride, and flour LS9c for various sponge times.   Small circles, no-time sponge; closed circles, 1 hour; double circles, 2 hours; and large circles, 3 hours of sponge time.

ammonium chloride dough than in the control dough at the time of their respective peaks.   Nonetheless, the ammonium chloride peak is higher.   These facts will have to be taken into consideration in any attempt to explain the ammonium chloride effect.

Commercial formula—80% sponge—flour LS11.—This flour is ana- lytically akin to LS9c.   It differs from that flour, however, in its sponge-curve characteristics as is evident from Figure 1.   The leveling- off point occurs somewhat later at about 4 hours.   Figures 6 and 7 represent the dough curves, doughs having been produced from sponges of varying age.   It is evident that even the 27-hour dough curve is superimposible upon the 4-hour curve for the first 2 hours of dough

fermentation. At the 2-hour point in dough fermentation, however, according to the baking schedule already outlined, our loaf would have been out of the oven some 25 minutes.

The three preceding cases, then, may be considered proof of the initial argument, particularly since several dozen other isolated cases have been studied without unexpected disagreement. This restriction placed upon the general principle may occasionally manifest itself,

Fig. 5. Dough curves via basic formula, 50% sponge, 0.05% ammonium chloride, and flour LS9c for various sponge times. Closed circles, 3½ hours; open circles, 5 hours; and double circles, 5½ hours of sponge time.

either by a slight lowering or rising of the dough curve as the sponge time is increased beyond the minimum sponge time involved. For example, the presence of papain (in the order of 0.002%–0.004%) may indirectly stimulate a positive response very likely because of the induced progressive increase in amino nitrogen. A negative response of magnitude varying with the substrate may be explained by a differential in rate of inhibition or of destruction of zymase. Notwithstanding such minor fluctuations it certainly appears that if fermentation
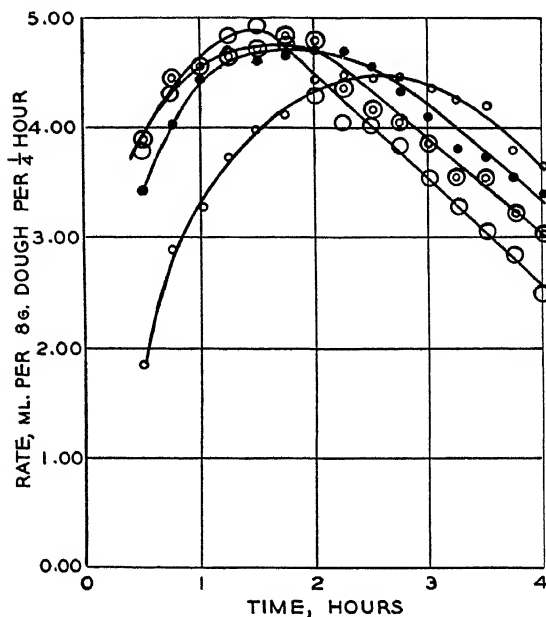
Fig. 6.   Dough curves via commercial formula, 80% sponge and flour LS11 for various sponge times.   Small circles, no-time sponge; closed circles, 1 hour; double circles, 2 hours; and large circles, 3 hours of sponge time.
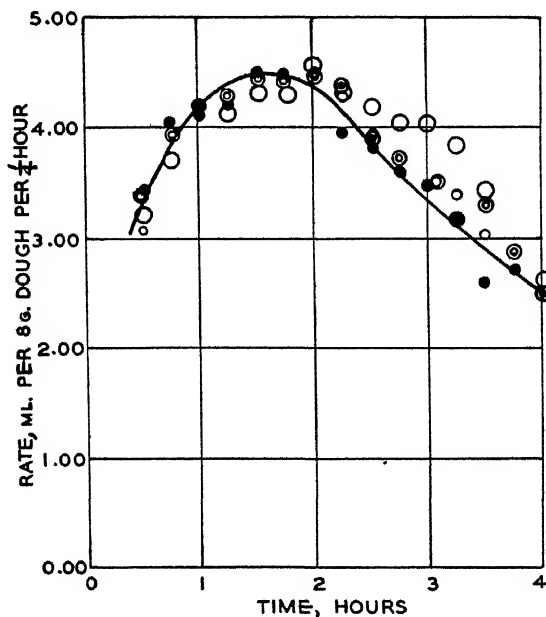
Fig. 7.   Dough curves via commercial formula, 80% sponge and flour LS11 for various sponge times.   Closed circles and solid line, 4 hours; small circles, 5 hours; double circles, 6 hours; and large circles, 27 hours of sponge fermentation.

time is a variable to be considered, the straight-dough cannot possibly compare in utility to the sponge-dough procedure.

Separation of Gas Production as Variable in Baking Test

One suggestion has already been given, namely, the use of an excess of maltose rather than sucrose plus about 0.07% ammonium chloride in the basic A.A.C.C. procedure and proofing during the third hour. Though this prove to be satisfactory for the purpose, we are not in a position at this time to discuss its application to the sponge-dough method. But it is the sponge-dough method which has been shown to be of more general utility. Hence we will consider two other attacks. The first will be the more direct and the more exact, but unfortunately somewhat less practical in application. The second may prove as exact as the baking test with which it is to be associated, if not more so, and therefore may be considered precise enough for the purpose.

The method of variable proof time.—This method is based on the assumption that small variations in dough time are of no significance except insofar as they affect gas production. We consider the assumption justified in view of the fact that 80% of the flour will already have received more than $3\frac{1}{2}$ hours of fermentation in the sponge. Further, the constant factor of 150/8 relating gas-production data and baking data will be absorbed in any other constants to appear, and, though in no sense being neglected, need not prevent us from directly employing gas-production data obtained via 8.0-g. aliquots.

Two conditions should be satisfied in the proper elimination of gas production during proof as a variable: (1) gas production during proof must be common, and (2) the rate of gas production upon completion of proofing must also be common from flour to flour and supplementary ingredient to supplementary ingredient. Both of these conditions cannot in general be simultaneously fulfilled without requiring the dough time to undergo relatively large variations. Hence, one must here make his choice between fixing the factor of dough time or fixing the factor of the rate of gas production upon entrance to the oven. The former condition, however, is met simply by determining the dough curve prior to baking, selecting a standard interval of time from mixing of dough to entrance in oven, and computing the time at which molding must occur in order to permit an arbitrarily selected and standardized amount of gas to be produced during proofing. Or more simply, employ a standard dough time and proof to a common level of gas production as determined by simultaneous study of an aliquot.

Both conditions can at times be satisfied with the occasional sacrifice of fixed dough time by the following technique. Here the dough

curve must be obtained prior to baking.   Further, besides standard-
izing the amount of gas to be produced one must also standardize the
rate to be desired at the completion of proofing.   For our purpose we
standardize gas production during proof at 17.00 ml. and rate at
entrance to oven at 3.75 ml. per quarter hour.   Then from the dough
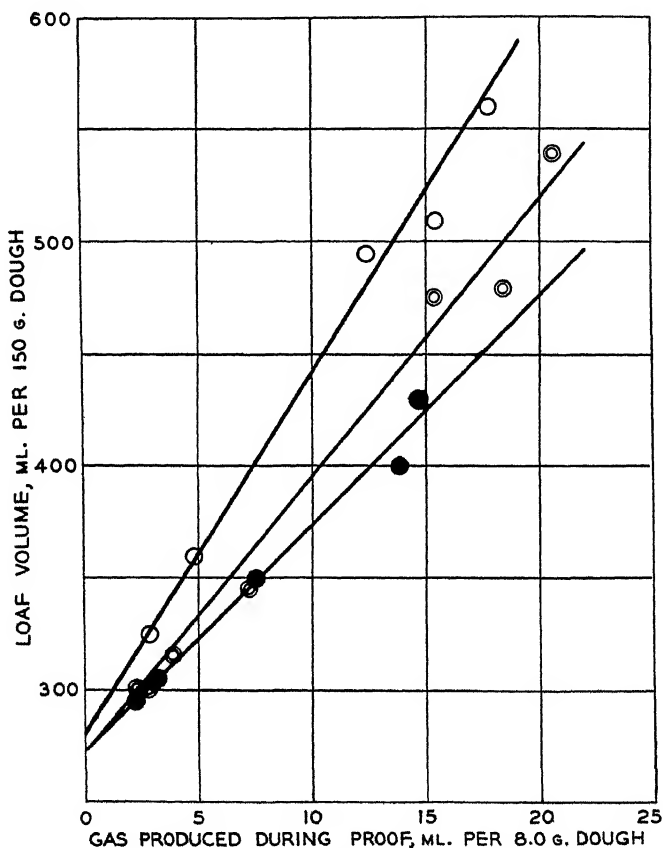
Fig. 8.   Standard A.A.C.C. procedure, primitive formula, fermentation time variable in ½-hour
steps beginning at 1 hour.   Open circles, flour Lab3; closed circles, flour Lab2; and double circles,
flour LS9c.

curves we determine the time required for the rate on its decline to
reach 3.75 ml. per quarter hour.   This time is to constitute the period
from mixing of dough to entrance in the oven.   The duration of
proofing is calculated from the dough curve so that 17.00 ml. of gas
is produced.   For example consider the 4-hour curve in Figure 7.
The required rate is reached in 2½ hours.   It is evident that if molding
were done at the 1-hour-and-29-minute point, 17.00 ml. of gas would

be produced in the 1-hour-and-1-minute interval between the two times.

As can be seen, this method is cumbersome and requires that complete gas-production data be available before the baking test is
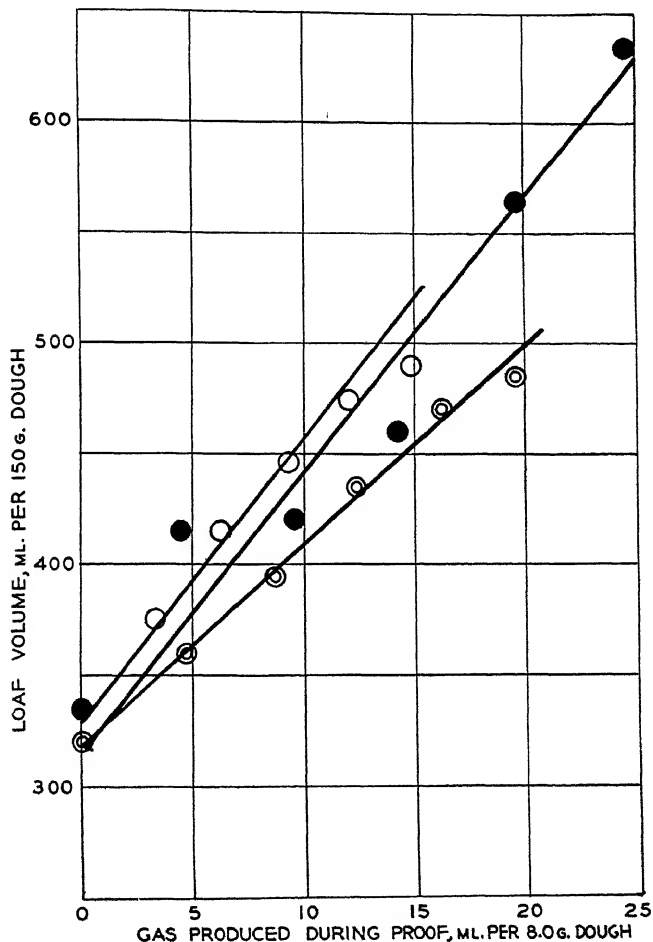
Fig. 9. Sponge-dough procedure, basic formula, 3½-hour sponges, proof time variable in ½-hour steps beginning at zero proof. Closed circles, flour Lab37; open circles, flour 7540; double circles, flour 101837.

attempted. Another objection rests with the fact that variable proof times are not in harmony with a simple schedule of procedure.

The method of constant proof time.—We do not here attempt to control gas production during proof; rather we determine the functional relationship between it and loaf volume. That is, we hope to

predict the loaf volume that would be obtained were 17.00 ml. of gas produced during proof from data acquired via a fixed time schedule. Hence, only loaf volumes and not other characteristics are to be separated from gas production, though we may here indicate that our findings concerning the variation of internal characteristics with moderate changes in proofing time are not such as to predict difficulties in interpretation.   It is this technique which will be employed by us in future communications.

The theory of the method is based on the premise that loaf volume is a continuous function of gas produced during proof, notwithstanding the fact that an occasional point as determined may indicate discontinuity by deviating from the best continuous line through all observed points by more than the apparent experimental error of the baking test as estimated from replicate determinations at that time. This is justified at least in part by our repeated observation that two pairs of loaves prepared several hours apart often differed in average volume by 20 to 25 ml. though the volume spread between loaves in each pair was no greater than 5 or 10 ml.

In Figures 8 and 9 we present loaf volume as a function of gas produced during proof for six flours, the analytical characteristics of which are indicated in Table I.   These figures consider the two possible

TABLE I

ANALYSES OF FLOURS STUDIED, CORRECTED TO 15% MOISTURE BASIS

Flour	Protein	Ash	Retention constant (average)
	%	%	
Lab2	9.5	0.48	7.0 [1]
Lab3	10.6	0.40	13.6 [1]
Lab37	16.7	0.84	11.2 [2]
LS9c	11.9	0.40	9.6 [1]
LS11	11.9	0.40	——
7540	8.2	0.38	14.9 [2]
101837	10.9	0.41	9.2 [2]

[1] Primitive formula.
[2] Basic formula.

ways of varying gas production during proof, namely, that of varying the rate of gas production and proofing for a constant time, or that of maintaining a relatively constant rate of gas production and varying the proof time.   Under both of these conditions sensibly linear behavior is observed.   The results of Figure 8, however, must be considered in part fortuitous since linearity can be expected under these circumstances only if the physical properties of the dough do not vary with fermentation time.   That this in general is not true is evident upon consideration of unsound flours or those treated with potassium bro-

mate, proteolytic enzymes, or other agents that affect these physical properties in a manner depending upon the duration of fermentation. It seems, therefore, that the arbitrarily selected flours here employed do not fall into any of these categories; rather they must be considered sound flours, and one must conclude that the physical properties of such flours insofar as loaf volumes are concerned display excellent stability towards changes in fermentation time. This in fact has been corroborated by the sponge-dough method already described by preparing loaves from 3-hour and 13-hour and even 24-hour sponges without finding any significant changes in loaf volumes. This is exemplified in Table II.

TABLE II

Loaf Volume, Gas Produced during Proof, and Loaf Volume Corrected to 17.00 ml. of Gas during Proof for Flour LS9c as a Function of Sponge Time—Commercial Formula

Sponge time	Loaf volume	Gas during proof	$V_{st} = 320 + 17.00K$
hrs.	*ml. per 150 g. dough*	*ml. per 8 g. dough*	
3	515	14.70	545
5	530	14.20	570
13	500	13.40	550

The results of Figures 8 and 9 may therefore be expressed by the linear equation:

$$V = V_0 + K\bar{R}_P t = V_0 + K V_P \qquad (2)$$

in which V represents the loaf volume obtained after t quarter hours of proofing, V_0 represents loaf volume at either zero proof or zero rate of gas production during proof, and $\bar{R}_P$ the average rate of gas production during the proofing period. V_P therefore equals $\bar{R}_P t$, the volume of gas produced during proof. K may be called the *retention constant*. It is apparent that V depends upon gas production during proof and upon all dough characteristics other than gas production, whereas V_0 may be considered independent of the former and substantially independent of the latter. By substantially independent we do not imply the absence of causal relationship between V_0 and flour characteristics; rather we imply that this relationship may be neglected in comparison to the more significant connection of V and flour characteristics. This assumption may be expected to approach exactness as the removal of residual gas by molding approaches completeness. Hence it may be expected to hold more closely for mechanical than for hand molding.

The estimation of V_0 can be made either from plots such as those under discussion or by merely baking doughs immediately out of the

molder. Seventeen independent determinations under the conditions of zero proof of Figure 9 for five flours and both the straight-dough and the sponge-dough basic-formula procedures lead to $V_0 = 319 \pm 11$ ml. This value is some 40 ml. higher than that of the V_0 obtained from data acquired under the straight-dough, primitive-formula, constant-proof-time procedure of Figure 8. We will now attempt both to interpret this and give theoretical justification for equation No. 2.

We write:

$$V = v_0 + \frac{150}{8} \frac{T_i}{T_{st}} \frac{P_{ex}}{P_i} \left[\int_0^{t_P} (R_P - R_L)dt + \int_{t_P}^{t_s} (kR_P - R_L)dt \right] \quad (3)$$

in which v_0 represents the loaf volume that would be obtained after any period of proofing if both the rate of gas production during this time and during the three or four minutes from t_P, the time of entrance in oven, to t_s, the time of setting of the loaf, were zero.

It is here assumed that retention characteristics may be neglected and that responses due to water vapor and dissolved gases are constant and independent of flour characteristics. With high-compression mechanical molding, the additional response due to entrapped carbon dioxide and air may also be included in v_0. T_i and T_{st} respectively represent the average loaf temperature at t_s and the standard loaf temperature upon entrance in the oven (approximately 303°K.). Similarly, P_i and P_{ex} signify the average pressure within the loaf at t_s and the external atmospheric pressure respectively. The integral between the limits of 0 and t_P equals the volume of gas retained during proof, since R_P and R_L are taken as the rates of gas production and of leakage respectively in milliliters at T_{st} and P_{ex} per 8.0 g. dough per quarter hour. In like fashion, the second integral denotes the volume of gas retained under the same standard conditions in going from t_P to t_s. The unit of time, of course, is the quarter hour. k is the temperature coefficient of the rate of gas production; that is, it is the ratio of R_P at $\bar{\bar{T}}_i$ to that at T_{st}, $\bar{\bar{T}}_i$ being defined as an internal loaf temperature averaged not only through space but over the time interval t_P to t_s.

Equation No. 3 may be simplified as follows:

$$V = v_0 + \frac{150}{8} \frac{T_i}{T_{st}} \frac{P_{ex}}{P_i} \left[(\bar{R}_P - \bar{R}_L)t_P + (k\bar{\bar{R}}_P - \bar{\bar{R}}_L)(t_s - t_P) \right] \quad (4)$$

in which the single bars above the symbols represent an averaging over the time range 0 to t_P. Similarly the double bars above the indicated symbols represent an averaging of the variables concerned over the time range t_P to t_s.

We now make an assumption which as will be seen is justified by the experimental evidence of Figures 8 and 9; namely, that:

$$\bar{R}_L = K'(\bar{R}_P - \bar{R}_L) \tag{5}$$

and that:

$$\bar{\bar{R}}_L = C'(k\bar{\bar{R}}_P - \bar{\bar{R}}_L). \tag{6}$$

That is, we take the rate of leakage as being proportional to the rate of increase of gas within the dough mass. Therefore:

$$\bar{R}_L = \frac{K'\bar{R}_P}{1 + K'} \tag{7}$$

and:

$$\bar{\bar{R}}_L = \frac{C'k\bar{\bar{R}}_P}{1 + C'}, \tag{8}$$

hence:

$$(\bar{R}_P - \bar{R}_L) = \bar{R}_P\left(1 - \frac{K'}{1 + K'}\right) = K''\bar{R}_P \tag{9}$$

and:

$$(k\bar{\bar{R}}_P - \bar{\bar{R}}_L) = k\bar{\bar{R}}_P\left(1 - \frac{C'}{1 + C'}\right) = C''k\bar{\bar{R}}_P. \tag{10}$$

These relationships are inserted in equation No. 4 to yield:

$$V = v_0 + \frac{150}{8}\frac{T_i}{T_{st}}\frac{P_{ex}}{P_i}[(K''\bar{R}_P t_i) + C''k\bar{\bar{R}}_P(t_s - t_P)]. \tag{11}$$

The significance of the *retention constant* now becomes clear, for placing:

$$\frac{150}{8}\frac{T_i}{T_{st}}\frac{P_{ex}}{P_i}K'' = K \tag{12}$$

and:

$$\frac{150}{8}\frac{T_i}{T_{st}}\frac{P_{ex}}{P_i}C'' = C, \tag{13}$$

we obtain:

$$K = \frac{150}{8}\frac{T_i}{T_{st}}\frac{P_{ex}}{P_i}\left(\frac{\bar{R}_P - \bar{R}_L}{\bar{R}_P}\right). \tag{14}$$

Equation No. 11 may be simplified to:

$$V = v_0 + Ck\bar{\bar{R}}_P(t_s - t_P) + K\bar{R}_P t. \tag{15}$$

We need now only place:

$$V_0 = v_0 + Ck\bar{\bar{R}}_P(t_s - t_P) \tag{16}$$

and equation No. 2 has been deduced. Furthermore, the difference of 40 ml. between the V_0 estimated from Figure 8 and that from Figure 9 is explained merely by the lower rate of gas production in the

oven for the former case in the range of low loaf volumes. It is evident that, since the loaf-volume intercepts of Figure 8 must vary with the rate of gas production at entrance to oven, the relationship between V and V_P should certainly be curvilinear. But it is also evident that if one neglects the points at low values of V_P (equivalent under the fixed-time schedule employed to neglecting points at low R_P), then an intercept of 320 ml. serves as well for the remaining points as does an intercept of 280 ml. That is, the baking test, at least in our hands, is not sufficiently precise to demonstrate the curvilinearity.

TABLE III

LOAF VOLUMES, GAS PRODUCED DURING PROOF, RETENTION CONSTANTS CALCU-
LATED BY EQUATION NO. 20, AND PREDICTED LOAF VOLUMES FOR THREE
FLOURS—PRIMITIVE FORMULA, STRAIGHT-DOUGH PROCEDURE—
AS A FUNCTION OF FERMENTATION TIME

Fermentation time (hrs.)	Loaf volume (V)	Proof gas (V_P)	K [1]	Loaf volume (calc.)	Difference from exp.
		FLOUR LAB2			
1	400	13.9	6.3	409	9
1½	430	14.7	7.6	417	13
2	350	7.5	7.5	346	4
2½	305	3.2	7.6	302	3
3	295	2.4	7.5	294	1
		Average	7.0 ± 0.7		±6
		FLOUR LAB3			
1	510	15.5	12.5	528	18
1½	560	17.9	13.2	568	8
2	495	12.5	15.0	478	17
2½	360	4.8	15.8	349	11
3	325	2.9	15.8	318	7
		Average	13.6 ± 1.0		±12
		FLOUR Ls9c			
1	480	18.45	8.4	502	22
1½	540	20.60	10.1	530	10
2	475	15.25	10.4	462	13
2½	345	7.35	7.2	363	18
3	315	3.65	9.3	316	1
3½	300	2.85	7.2	305	5
4	300	2.55	8.6	302	2
		Average	9.6 ± 0.8		±11

[1] Average values of K are computed only from data wherein V is greater than 400 ml. in order to avoid large percentage errors arising from low volumes.

An interesting consequence of equation No. 15 lies in the possibility of determining at least the order of magnitude of k, the temperature coefficient of gas production from baking-test and gas-production data during proof. To do this, we first assume that C does not differ so far from K that one may not be replaced by the other. We may further take $(t_s - t_P)$ approximately equal to 3.5/15 quarter hour.

In addition $\bar{\bar{R}}_P$ may as a first approximation be placed equal to V_P/t_P in which $V_P = \bar{R}_P t_P$. Also, for the data of Figure 8, $t_P = 55/15$ quarter hours. Hence equation No. 15 may be rewritten:

$$V = v_0 + \frac{3.5}{55} K k V_P + K V_P. \tag{17}$$

Upon consideration of the open circles of Figure 8, we find $V = 560$ ml. for $V_P = 17.8$ ml. For this relatively large value of V_P we take $V_0 = 320$ ml. and calculate $K = (560 - 320)/17.8 = 13.5$. For this

TABLE IV

LOAF VOLUMES, GAS PRODUCED DURING PROOF, RETENTION CONSTANTS CALCU-
LATED BY EQUATION NO. 20, AND PREDICTED LOAF VOLUMES FOR
THREE FLOURS—BASIC FORMULA, SPONGE-DOUGH PRO-
CEDURE—AS A FUNCTION OF PROOF TIME

Proof time (hrs.)	Loaf volume (V)	Proof gas (V_P)	K	Loaf volume (calc.)	Difference from exp.
		FLOUR LAB37			
0	335	0.00	—	325	10
1/4	415	4.50	21.1 [1]	370	45 [1]
1/2	420	9.65	10.1	431	11
3/4	460	14.35	9.6	484	24
1	565	19.65	12.3	544	11
1 1/4	635	24.65	12.6	600	35
			Average 11.2 ± 13		±18
		FLOUR 7540			
0	320	0.00	—	305	15
1/4	375	3.30	20 9	355	20
1/2	415	6.25	17.6	398	17
3/4	445	9.40	14.9	445	0
1	475	12.05	14.3	483	8
1 1/4	490	14.90	12.6	525	35
			Average 14.9 ± 1.4		±16
		FLOUR 101837			
0	320	0 00	—	315	5
1/4	360	4.50	8.9	361	1
1/2	395	8.75	8.8	398	3
3/4	435	12.55	9.5	431	4
1	470	16.35	9.5	465	5
1 1/4	485	19.70	8.7	494	9
			Average 9.2 ± 0.4		± 5

[1] Eliminated from average.

same flour at $V = 325$ ml., $V_P = 3.0$ ml. For this relatively small value of V_P, we take $V_0 = 280$ ml. and calculate $K = (325 - 280)/3.0 = 15.0$. Hence, 14.3 serves as an average estimate of K for the flour under consideration. But:

$$\left(\frac{3.5}{55}\right)(14.3)(17.8)k - \left(\frac{3.5}{55}\right)(14.3)(3.0)k = 40.$$

We here find that the temperature coefficient of gas production, in going from the standard temperature of 303°K. to an internal loaf temperature averaged not only through the dough mass but over the time interval between entrance in oven and setting lies in the vicinity of 3.0.   That this value is completely rational is proved by estimating k by other means.   For example, Bailey and Munz (1938) have shown that the temperature of setting lies between 50°C. and 60°C.   Though their data are not strictly applicable to our conditions of working, we may very likely with less crude assumptions than have already been made take 55°C. as a tentative value.   That is, the loaf temperature increases some 25°C. from the time of entrance to oven to the time of setting.   Half of this value, 12.5°C., may be considered the average rise, wherein averaging is done over time.   Hence, we must calculate the temperature coefficient for the range 303°K. to 316°K.

But:

$$\log_e k = \frac{E(316 - 303)}{R(316)(303)} \tag{18}$$

in which k is the desired temperature coefficient, R is the gas constant in calories per degree, and E is the energy of activation.   11,400 calories has been indicated as an average value of the energy of activation for enzymatic reactions.[9]   We find upon calculation that $k = 2.17$. This agreement, if not accidental, is certainly remarkable.

Therefore, in view of the fact that V_0 varies only about 40 ml. and that variations in C and K affect this value in a secondary manner as compared to the effect of K upon the remaining terms of equation No. 15, we may take $C = 15$ and rewrite equation No. 15 for the 55-minute proof period:

$$V = v_0 + 3.0 V_P + K V_P \tag{19}$$

in which $v_0 + 3.0 V_P = V_0$.   But we may take $V_0 = 280$ for low values of V_P, namely about 3.0 ml.   Therefore we may assume v_0 to be in the neighborhood of 270 ml.   We have finally:

$$V = (270 + 3.0 V_P) + K V_P \tag{20}$$

or for the sponge-dough procedure, in which V_P generally lies in the range 12 ml. to 20 ml.:

$$V = 320 + K V_P \tag{21}$$

with a maximum error in V_0 of about 10 ml.   In Tables III and IV are given the pertinent data of Figures 8 and 9 respectively with loaf volumes calculated by equation No. 20.   In applying this equation to

[9] See page 230 of Respiratory Enzymes by the University of Wisconsin Biochemists, 1939—Burgess Publishing Company, Minneapolis, Minn.

the data acquired with variable proof time, it is obvious that V_P in parentheses must be calculated to a 55-minute basis, while V_P outside the parentheses must be the value determined for the given proof time.

One may here indicate that whereas V_0 and V may be expected to vary with different operators and with different equipment, K should prove relatively independent of such factors. Hence if V_0 and V_P are standardized at say 320 ml. and 17.00 ml., respectively, calculated loaf volumes obtained by different workers upon the same ingredients may well show more agreement than heretofore has been possible. That is, each worker first is to obtain his own V_0. He will then determine V and V_P for a given flour and calculate K by equation No. 20. A new standard V_{st} may be calculated by means of:

$$V_{st} = 320 + 17.00K. \tag{22}$$

Loaf Volume at Standard Gas Production

From Table I it is seen that no correlation seems to exist between K and protein content. Though variations in gas production during proof are eliminated, it appears nevertheless that loaf volume remains a function of more than one variable. This is demonstrated more explicitly by equation No. 14 where we find K and therefore V_{st} to be a function of the independent flour variables $\bar{R}_P$, $\bar{R}_L$ and P_i. Loaf volume measures a composite effect, and for the moment it is only through experience and strained interpretive processes in which dough and loaf characteristics serve as guides that loaf volumes may be taken to measure flour utility or *strength*. That fairly high correlation coefficients between loaf volume and protein content have been found in the past can be explained at least in part by the data of Tables I, III, and IV. It is seen that gas production during proof at corresponding times for corresponding conditions is a more definite function of protein content than is either K or V_{st}.

In Table V are recorded the maximum values of V_P obtained for a given flour and its protein content. The correlation between these

TABLE V
MAXIMUM VALUES OF V_P AS A FUNCTION OF PROTEIN CONTENT

Condition	Flour	Protein	Gas during proof
			ml.
Straight-dough, primitive formula, variable fermentation time. Proof time constant.	Lab2	9.5	14.7
	Lab3	10.6	17.9
	LS9c	11.9	20.6
Sponge-dough, basic formula, 3½-hr. sponge time. Proof time variable.	7540	8.2	14.90
	101837	10.9	19.70
	Lab37	16.7	24.65

variables becomes even more evident by study of Figure 10 in which one is plotted against the other for some fifteen flours. Several comments concerning the data of this figure are in order. First there was no observable relationship between 4-hour gas productions (primitive
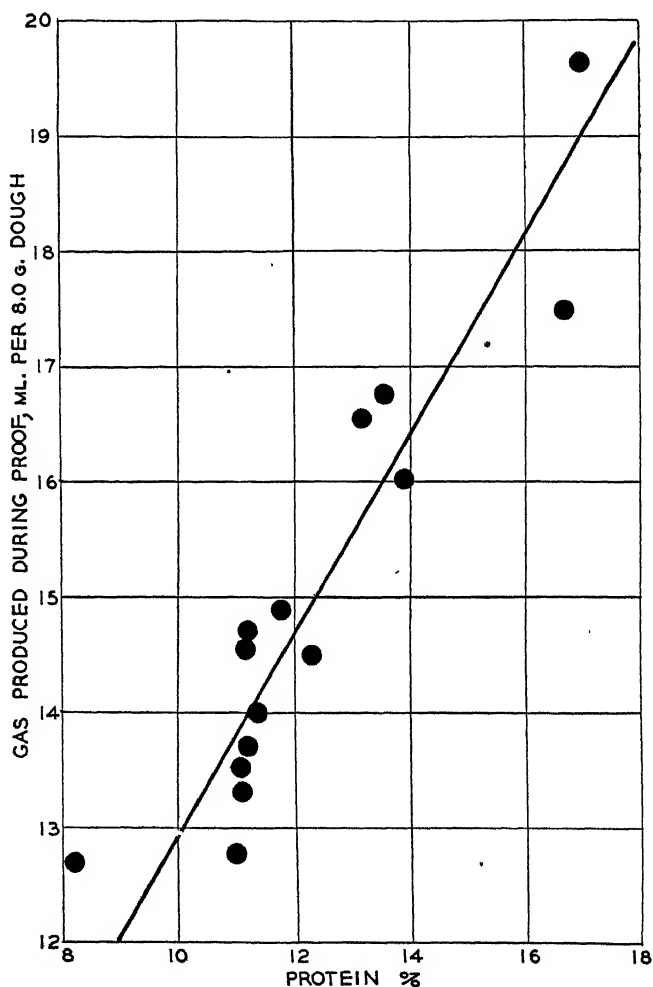
Fig. 10. Standard A.A.C.C. procedure, basic formula. Gas production during proof as a function of protein content.

formula) and protein content. Second, the variations in gas production during proof for these flours may be explained by four different hypotheses: (1) variations in thiamin, (2) variations in the *Z* factor of von Euler, (3) variations in the *M* factor of Blish and Sandstedt, or

(4) variations in amino nitrogen. Of these four, one can more readily visualize a correlation between amino nitrogen and protein content.

The problem of flour *strength* or that of gluten *quality* thus resolves itself into the interpretation of the relative significance of P_i and $\bar{R}_L$. Measurements of so-called *retention capacities* obviously cannot separate these factors; at best when taken in conjunction with gas-production data they may measure some average value of $P_{ex}/P_i\bar{R}_L$. One must also realize that while P_i may not in general be expected to differ greatly from P_{ex}, $\bar{R}_L$ itself is very likely a sensitive function of P_i. Therefore slight differences in P_i may prove of great importance in the proper interpretation of V_{st}. Furthermore, it seems likely that P_i may be a relatively simple function of the amount of force required to extend a unit block of dough a unit distance. It is not unlikely, therefore, that properly conducted extensometric measurements might ultimately lead to the desired separation of these dependent variables.

In conclusion, we may point out a field of at least qualitative utility in the relationships that have been derived. We have shown that both the retention constant and the standardized loaf volume are functions of the rate of leakage and of the internal pressure. For a given V_P, K, or V_{st} must evidently increase with decrease in R_L or decrease in P_i. We may for example apply these considerations to a flour possessing a positive potassium bromate response. Mere familiarity with the general effect of potassium bromate on dough characteristics is sufficient to indicate an increase in P_i. Hence, in order to obtain a positive volume response, it is evident that $\bar{R}_L$ must undergo a greater decrease than is apparent from loaf volume alone. Similarly, it can be shown that positive volume responses to malt preparations are generally greater than can be expected from only increases in gas production during proofing. Here we may qualitatively conclude that the increase in K or V_{st} is due primarily to a decrease in P_i rather than to a decrease in $\bar{R}_L$. It follows that a large loaf volume may at times be an indication of weakness as well as of strength. We have observed instances of this in the study of certain supplementary ingredients which, though almost invariably producing a positive volume response under usual laboratory conditions, produced a distinct negative response under commercial conditions of pound and a half loaves and forced steam in the oven.

Summary

Criteria for the design of a baking test to be used in the study of flour and supplementary ingredients are discussed. With these as a basis a sponge-dough procedure and primitive, basic, and commercial formulas are selected. Several methods for the separation of gas

production as a variable are described.   One of these is advocated as particularly practical in application and worthy of continued study. It is shown, however, that loaf volumes obtained even under such conditions must be subjected to interpretation by an experienced worker before being of practical utility.   Such loaf volumes remain a function of at least two principal variables, the rate of leakage of gas and the gas pressure developed within the dough.   These variables may perhaps be separated one from the other by extensometric measurements.

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THE EFFECT OF LOW TEMPERATURE IN PREVENTING DAMAGE TO WHEAT STORED WITH HIGH MOISTURE CONTENT [1]

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It was shown by Swanson and Fenton (1932) that the fundamental cause of damage to wheat stored in farm bins is high moisture. This will favor growth of molds, and their biological activities as well as those of the wheat will generate heat. Since increase in temperature will also increase these activities, the process is self-accelerating and, if not stopped, will proceed until enough heat is generated to cause serious damage to the qualities of the wheat. Thus, while heat is the direct cause of the damage, the real cause was the high moisture content at the time of storage.

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[1] Contribution No. 70, Department of Milling Industry.

That wheat may be stored 11 years in a farm bin without heating, and consequently remain undamaged, was shown by A. F. Swanson (1939). The moisture content of this wheat was 11% at the time of removal from the bin and had probably never been higher than 12% since it was combined in northwest Kansas when the weather was dry. The milling and baking tests revealed no apparent damage and some of the seed produced a stand equal to that from new seed. The climate is cooler and dryer in northwest Kansas than further east. A small sample of wheat stored for about 20 years in the loft of a granary near Junction City, Kansas, was dead and the volume of the baked loaf was 1140 cc. as compared with 2090 cc. for the check (Swanson, 1938). The longer time was probably a factor, but the higher temperature was no doubt the principal factor causing this damage.

In a previous publication (Swanson, 1934) it was shown that damage resulted most rapidly in samples stored in the laboratory during the summer, less rapidly when stored at about 60° F., and least rapidly when stored outdoors in the winter. It was also shown that the access of air was an important factor in mold development and also in the development of rancidity or acidity in the wheat fat. This is estimated by determining the mg. of KOH neutralized per gram of fat which is obtained by extracting a ground portion of wheat with ether. This development of acidity in the fat did not take place unless the conditions were such that mold developed. Serious damage did take place in the absence of mold, but this did not show itself in increased fat acidity.

The object of the present investigations was to confirm some of the previous findings as well as to get additional information, particularly on the effect of low temperature. Since the effects of low temperatures cannot be determined except in conjunction with other factors, variation in the amount of moisture, air access, and duration of time were also included. These experiments extended over two seasons, 1938–39 and 1939–40, and wheat from each season's harvest was used. The previous work had shown (Swanson, 1934) that it made but little difference whether wheat was used which was high in moisture because of being cut before complete desiccation had taken place, or whether water was added to wheat which had once been dry. It was the amount of moisture in connection with the high temperature which determined the extent of damage.

Experiments in 1938–39

Decrease in viability.—Wheat was placed in a number of 4-ounce glass-stoppered bottles. Half the number were placed in the laboratory and half in a room at 41° F. At various intervals these were

tested for viability.   The most significant figures are given in Table I. There was no decrease in viability in the samples stored at 41° F. after 21 weeks, even when the moisture content was 20%.   When stored in the laboratory the viability of the 14% moisture wheat had notably

TABLE I

EFFECT OF MOISTURE AND TEMPERATURE ON THE RATE OF DECREASE IN VIABILITY

Moisture	Laboratory		Stored at 41° F.	
	Weeks old	Germination	Weeks old	Germination
%		%		%
10	18	92	18	91
12	18	94	18	92
14	18	51	18	93
16	9	0	21	91
18	6	1	21	88
20	6	0	21	90

decreased at the end of 18 weeks and those with higher moistures were dead at the end of 6 and 9 weeks.   This clearly indicates the efficacy of the lower temperature in preserving viability of wheat.   As will be shown later, a high percentage of viability indicates that the wheat has suffered no damage to its milling and baking qualities.

TABLE II

FLOUR YIELDS AND ASH CONTENT FROM SAMPLES STORED AT VARIOUS MOISTURE CONTENTS IN THE LABORATORY AND AT 41° F.

Moisture during storage	Laboratory temperature		Cold room 41° F.	
	Flour yield	Ash [1]	Flour yield	Ash [1]
%	%	%	%	%
Check [2]	75.3	0.41	77.7	0.40
10	75.4	0.41	76.0	0.40
12	76.2	0.42	76.8	0.40
14	78.5	0.44	75.8	0.39
16	77.2	0.44	77.0	0.41
18	78.9	0.48	77.2	0.42
20	78.9	0.51	77.5	0.43

[1] Calculated on the 15% moisture basis.
[2] The checks in this and following tables were from the original lot of wheat used in these experiments and were stored in tin cans with slip-on covers alongside each group of bottles.

Effects of temperature and moisture on milling and baking qualities. —To determine the effect of temperature and moisture on the milling and baking qualities 1800-g. portions of wheat were placed in gallon bottles and amounts of water added which were required to provide samples, respectively, of 10, 12, 14, 16, 18, and 20% moisture.   There

were two lots of bottles.   One lot was stored in the laboratory and another lot was stored in the room kept at 41° F.   The storage period was about seven months.   To get the samples uniformly dry at the end of the storage period, the wheat was emptied from the bottles into shallow paper boxes and exposed until air-dry, which was known by comparing the weights of the higher moisture samples with the lower. The milling was done on the Buhler mill.   The flour yields and the ash content on a 15% moisture basis of the straight flours are given in Table II.   The flour yields indicate the percentage obtained from a weighed amount of cleaned wheat and have thus a comparative value among the samples.   The flour yields were large without an unduly high ash.   In the laboratory-stored samples, however, there was a gradual increase in ash content parallel with the higher moistures during storage, showing some deterioration in milling properties.

The flours were baked after they had been stored in the mill room for about six weeks.   Two different formulas were used:

> Formula A   2% yeast, 1.75% salt, 6% sugar,
> 3% shortening

> Formula B   2% yeast, 1.75% salt, 6% sugar,
> 3% shortening, 6% dry milk solids,
> 3 mg. potassium bromate.

The absorption on the existing moisture basis and the mixing time were optimum as determined by observation.   Doughs were made from 200 g. of flour, divided into equal parts and fermented at 86° F., 105 minutes to first punch, 50 minutes to second punch, 25 minutes to third punch when the doughs were passed between the rollers, molded in the Thompson molder and formed.   The proof was for 55 minutes at 86° F.   The baking was done in a Despatch rotary oven.   The loaf volumes were the averages of duplicate loaves.   One set of loaves was used for scoring and one set for photographing.

The results of the baking tests are given in Table III.[2]   The flour moistures were uniform among each set of the samples.   That is, the flours from the wheats which had had the higher moisture percentages were no higher than those which had had the lower percentages during storage.   The variation within each group of samples was only 0.3%; however, the average for the laboratory-stored samples was 12.54 and for the samples stored at 41° F. it was 13.84.   Why the flours from the wheats stored at 41° F. should average over 1% higher in moisture than those stored at laboratory temperature was not apparent.   The

percentages of absorption were lowest in the samples stored with the highest moisture, the damage being thus reflected in loss of water-holding capacity.

The data in Table III show that the deterioration started with 16% moisture in the laboratory-stored samples, while in the samples stored in the cold room there was no deterioration until the moisture of the wheat during storage was 20%, and then it was slight. In fact the samples having 18% moisture stored in the cold room had as large a volume and as high a crumb score as any of the loaves.

TABLE III

BAKING RESULTS ON FLOURS FROM WHEATS STORED AT VARYING MOISTURE CONTENT IN THE LABORATORY AND AT 41° F.

Moisture during storage	Laboratory				41° F.		
	Ab-sorp.	Loaf vol.	Crumb score	Color	Ab-sorp.	Loaf vol.	Crumb score
%	%	cc.			%	cc.	
				FORMULA A			
Check	61	615	64	Good	60	615	64
10	61	600	57	"	60	605	60
12	62	615	58	"	60	610	59
14	62	630	57	"	60	635	59
16	59	615	50	"	60	618	59
18	55	445	30	Muddy br.	59	658	78
20	52	335	10	" "	55	603	45
				FORMULA B			
Check	61	848	88	Good	60	798	94
10	61	795	87	"	60	815	85
12	62	785	88	"	60	798	89
14	62	770	88	"	60	830	89
16	59	570	40	Sl. brown	60	823	89
18	55	403	15	Muddy br.	59	820	94
20	52	295	10	" "	55	698	90

The addition of bromate and dry-milk solids in formula B greatly increased the loaf volumes and the crumb scores. The B formula served even better than formula A to differentiate between the damaged and undamaged flours. The cut sections of the loaves obtained from formula A are given in Figure 1 and for formula B in Figure 2. The numbers on the loaves indicate the moisture contents at which the wheats were stored. The upper rows are from the cold-storage samples and the lower rows from the laboratory-stored samples. The greater amount of damage from the higher moistures of the laboratory-stored samples is very evident in comparison with the samples stored in the cold room.

One difference between the wheat is these bottle samples and wheat in storage bins is that in the bottles the heat does not accumulate and
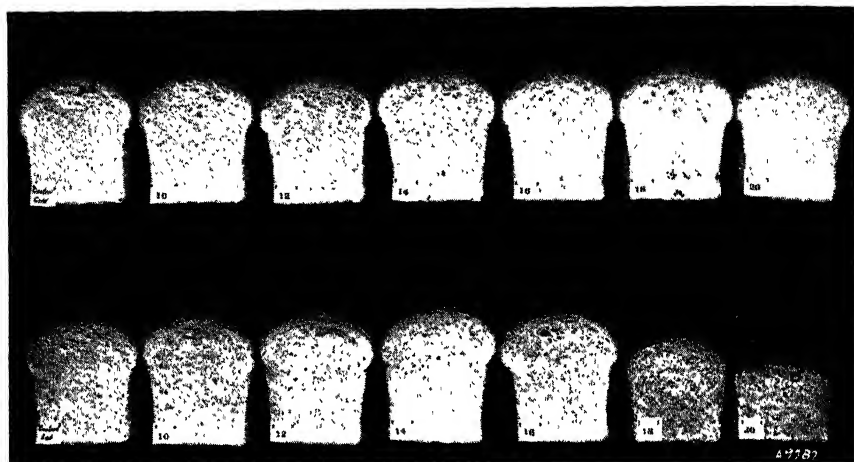
Fig. 1. Loaves baked with formula *A*.   Loaves in upper row are from cold storage, lower row from laboratory storage.   First loaves in each row are the checks; the numbers on the loaves indicate the moisture percentage of the wheat during storage.
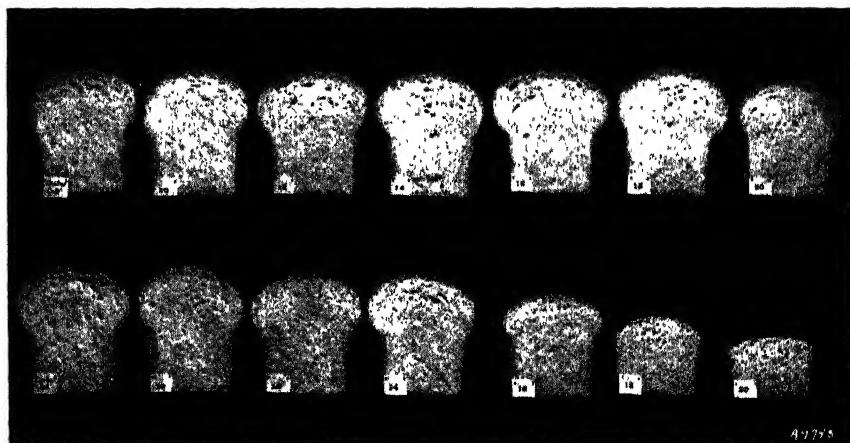
Fig. 2. Loaves baked with formula *B*.   Loaves in upper row are from cold storage, lower row from laboratory storage.   First loaves in each row are the checks; the numbers on the loaves indicate the moisture percentages of the wheat while in storage.

hence the temperature of the wheat is very near that of the room. In a bin the heat accumulates and hence the temperature will be much higher than that of the surrounding air. This is probably the reason for the much smaller damage in the 16% and higher moisture samples stored in the laboratory as compared with what would have been expected in a bin at these moistures.

Experiments in 1939–40

Besides temperature and moisture, the factors of air supply and duration of time were also included during 1939–40. The containers were gallon bottles as before except for the check samples which were stored in tin cans. The same range of moistures was used as before except that 15% moisture was also used because the critical range in bins has been found to be between 14% and 16% (Swanson and Fenton, 1933). There were two groups of bottles and three lots in each group, both for the laboratory and the cold room. One group was sealed so the wheat would be under anaerobic conditions after respiration had consumed the oxygen in the interkernel air space. The other group of bottles were closed with a one-hole rubber stopper in which was placed a wad of cotton. This would admit a limited air supply such as occurs in a deep bin. The general plan was to test one lot after three months, the second lot after six months, and the third lot after nine months.

Tenmarq wheat from the Agronomy Farm was used. This wheat had been exposed to several rains after it was ripe and hence had undergone a certain amount of weathering. The test weight was 57.9 and the moisture as received 13.3%. It was therefore necessary to air-dry a portion of this wheat so as to have low enough moisture for the 12% samples as well as for the checks. The experiment was started the latter part of July, 1939. The first lot of bottles was opened and tested the last part of October, 1939; the second lot, the last part of January, 1940; and the third lot the latter part of April, 1940.

The tests made at the end of each period, aside from check weighings, were the following: examination for soundness as revealed by mold, mustiness, or odor; test weights on the wet, air-dry, and scoured samples; specific gravity; proximate viability. Milling and baking tests were made only on the three and six months samples as the nine months samples were accidentally destroyed just before milling.

Results obtained.—The examination for soundness was made by visual inspection and smelling. The results of these examinations are given in Table IV. When air had access the unsoundness took the form of mold or mustiness and when it did not have access it took the form of a sour odor, and in a few cases this seemed sweetish. An

attempt to measure by titration the amount of sourness in the samples which had the sour odor gave erratic results. The slight mustiness which was noted at the end of three months in the two sealed samples, both the laboratory and cold stored, may have been the beginning of the process which resulted in the condition designated by "sour odor." Time is a factor in the development of this condition. At the end of nine months it was noted even with 15% and 16% moisture. Thus sealing and cold storage did not prevent the development of some of

TABLE IV

EXAMINATION FOR SOUNDNESS

Sample description [1]	3 months	6 months	9 months
c-check	Sound	Sound	Sound
c-air-12	"	"	"
c-air-14	"	"	"
c-air-15	"	"	"
c-air-16	"	"	"
c-air-18	Musty	Sl. sour odor	Musty
c-air-20	Some mold	Sl. moldy	"
c-sld-12	Sound	Sound	Sound
c-sld-14	"	"	"
c-sld-15	"	"	Sour odor
c-sld-16	"	"	" "
c-sld-18	"	Sl. sour odor	" "
c-sld-20	Sl. musty	Sweet	" "
1-air-check	Sound	Sound	Weevil eaten
1-air-12	Sound	Sound	Sound
1-air-14	"	"	"
1-air-15	Sl. musty	Sl. musty	Sl. musty
1-air-16	Musty	Musty	Very moldy
1-air-18	Very moldy	Very moldy	Very moldy
1-sld-12	Sound	Sound	Sound
1-sld-14	"	"	"
1-sld-15	"	"	"
1-sld-16	"	Sw. odor	Sour odor
1-sld-18	Sl. musty	Sweet-sour	Very sour

[1] Abbreviations: c = cold storage, sld = sealed, 1 = laboratory. Figures refer to moisture content while in storage.

those characteristics which are associated with damage. Such tests as these which are based on personal judgment are subject to considerable variation; however, the odor test, as will appear later, is more sensitive than several other tests.

The test weights were taken as soon as the wheat samples were emptied from the bottles, after they were air-dried, and also after they were scoured. The weights of the samples showed that all had dried to nearly 10% moisture and hence moisture variation was not a factor.

In studying the figures obtained it was noted that the length of storage did not have much influence on changes in test weights and, hence, the figures obtained from the three, six, and nine months storage were averaged for each moisture condition. These average test weights are given in Table V. In studying these data, it should be remembered that test-weight figures have considerable experimental errors regardless of the care taken in performing the test; hence only definite trends or larger differences have any meaning.

TABLE V

TEST WEIGHT AS INFLUENCED BY STORAGE CONDITIONS

	Cold—41° F.				Laboratory		
Description of sample	Wet	Air dried	Scoured	Description of sample	Wet	Air dried	Scoured
Air-check	58.1	58.1	60.6	Air-check	57.9	57.6	60.5
Air-12	57.3	57.1	60.4	Air-12	57.0	57.0	60.4
Air-14	57.5	57.3	60.7	Air-14	57.2	57.4	60.5
Air-15	56.3	56.6	60.3	Air-15	54.4	54.8	59.9
Air-16	56.3	56.8	60 2	Air-16	53.3	54.0	59.1
Air-18	53.4	55.1	59.8	Air-18	50.8	51.9	57.8
Air-20	52.3	54.1	58.9	—	—	—	—
Sealed-12	57.4	57.3	60.7	Sealed-12	57.3	56.9	60.6
Sealed-14	57.6	56.8	60.5	Sealed-14	57.6	56.6	60.2
Sealed-15	56.5	56.6	60.2	Sealed-15	55.7	56.4	60.3
Sealed-16	56.1	56.3	60.1	Sealed-16	54.8	56.0	59.9
Sealed-18	54.1	55.6	59.6	Sealed-18	52.9	55.3	59.4
Sealed-20	52.5	54.8	58.9	—	—	—	—

The samples stored in the cold decreased progressively in the test weights made before drying to the extent of nearly 5 pounds between the 12% and 20% moisture levels. After drying the decrease was nearly 3 pounds. That is, the increase in moisture content by addition of water to wheat will decrease the test weight in relation to the amount of water added, and this decrease will persist after redrying, but to a lesser degree. The amount of decrease in test weight due to wetting and redrying obtained in this experiment was not as great as in a former trial (Swanson, 1939). This was probably due to the partial weathering of this wheat before the experimental wetting. The test weights of the scoured samples were in all cases considerably larger than the unscoured because of the greater smoothness of the outer surface which resulted from the removal of the loose bran layers and which in turn would cause a closer packing. The decreases in test weight of the scoured samples were small with the progressive increase in moisture until the wetting had been 18% and 20%. This indicates that the smaller decreases in test weights of the dried unscoured samples were due mostly to the roughening of the outer bran layers. The

larger decreases which occurred with the greater amounts of wetting and which to a considerable extent persisted after scouring indicated that the swelling which took place as a result of wetting persisted after drying.

The trends in the change of test weight in the samples stored in the laboratory were the same as for those stored in the cold but the decreases were larger. This was due to the development of mold which would cause an additional roughening of the bran coat and also the destruction of some of the wheat substance. In the scoured wheat the decreases in test weight started in several samples at 15% and 16% moisture and became progressively greater as the molds had been more evident.

TABLE VI

VIABILITY OF STORED SAMPLES

Cold storage				Laboratory storage			
Description	3 mos.	6 mos.	9 mos.	Description	3 mos.	6 mos.	9 mos.
Check	Good	Good	Good	Check-air	Good	Good	Weevil eaten
Air-12	"	"	"	Air-12	"	"	Good
Air-14	"	"	"	Air-14	"	"	Very poor
Air-15	"	"	"	Air-15	Poor	Dead	Dead
Air-16	"	"	Fair	Air-16	Dead	Dead	"
Air-18	"	"	"	Air-18	"	"	"
Air-20	"	Fair	"	—	—	—	—
Sealed-12	"	Good	Good	Sealed-12	Good	Good	Good
Sealed-14	"	"	"	Sealed-14	"	Fair	Dead
Sealed-15	"	"	"	Sealed-15	Very poor	Dead	Poor
Sealed-16	"	"	"	Sealed-16	Dead	"	Dead
Sealed-18	"	"	"	Sealed-18	"	"	"
Sealed-20	"	"	"	—	—	—	—

Proximate viability.—The test for viability did not include kernel counts. Weighed amounts, estimated to be about 100 kernels, were placed between blotters, wetted, and placed in a germinator. The examinations by inspection were made after 4 and 7 days. Those in which nearly all kernels had vigorous sprouts were graded good. Those in which about half the kernels had sprouted were graded fair. The difference between fair and good could be only a proximate estimate. Some which had very few short sprouts were graded poor or very poor. Those that showed no signs of germination were designated dead. The results of this examination are given in Table VI. All the sealed samples in cold storage had good germination. Of those under aerobic conditions four were graded fair, namely: the 20% moisture after 6 and 9 months and the 16% and 18% moisture after 9 months. None in cold storage was dead.

For the samples stored in the laboratory, the decreases in viability started with 14% moisture for the 9 months and with 15% for the 3 and 6 months. There were 12 which had good germination, 5 poor to fair, and 16 were dead. Thus both duration of storage and amount of moisture were factors in the decrease in viability. The outstanding fact is the difference between the cold and laboratory storage in preserving the viability.

Flour yield and ash content.—The milling was done on the Buhler mill and at a constant setting of the rolls so as to obtain a uniform extraction as far as possible. The data on flour yields and ash content are given in Table VII. The percentages of flour yields are based on

TABLE VII

FLOUR YIELD AND ASH CONTENT

	Cold Storage				Laboratory Storage			
	3 months		6 months		3 months		6 months	
Description	Yield	Ash	Yield	Ash	Yield	Ash	Yield	Ash
	%	%	%	%	%	%	%	%
Check	76	0.45	71	0.38	76	0.50	73	0.41
Air-12	74	0.45	72	0.39	76	0.52	72	0.39
Air-14	73	0.46	74	0.37	77	0.52	73	0.39
Air-15	75	0.45	73	0.38	76	0.51	71	0.45
Air-16	73	0.45	73	0.38	75	0.49	75	0.50
Air-18	72	0.45	75	0.41	77	0.57	77	0.60
Air-20	73	0.42	72	0.42	—	—	—	—
Sealed-12	73	0.42	76	0.41	75	0.47	75	0.42
Sealed-14	76	0.45	76	0.41	74	0.43	75	0.45
Sealed-15	75	0.42	75	0.42	76	0.48	75	0.43
Sealed-16	73	0.42	74	0.42	75	0.51	74	0.51
Sealed-18	75	0.48	75	0.45	77	0.59	75	0.42
Sealed-20	76	0.50	74	0.45	—	—	—	—

the amount of wheat milled and do not show any definite trends which can be related to the storage conditions. The moisture percentages of the flours were very uniform, most samples ranging from 13.4 to 13.7, with an extreme spread of 13.0 to 13.8. The average of all was 13.5%. The ash figures were corrected to the 15% moisture basis. In most samples, the ash is lower in the flour from the 6 months than from the 3 months storage, and there is also a trend toward an increase in ash for the higher moisture samples. This trend was greater for those stored in the laboratory due to the mold than for the samples stored in the cold which were not moldy.

Baking tests.[3]—The following formula was used in making the baking tests:

100 g. flour	1.5 g. salt
2 g. yeast	3 g. shortening
6 g. sugar	1 mg. potassium bromate

The details of performing the baking tests were the same as for the previous year. The most significant figures, loaf volumes and the average of the baking scores, are given in Table VIII. Figures are also given for the loaf numbers represented in Figures 3 and 4. A

TABLE VIII

BAKING RESULTS

	3 months storage						6 months storage						
	Cold			Laboratory			Cold			Laboratory			
Description	Vol. cc.	Score %	No. on photo	Vol. cc.	Score %	No. on photo	Vol. cc.	Score %	No. on photo	Vol. cc.	Score %	No on photo	Odor of dough
Check	733	87	7	725	86	1	725	87	7	731	87	1	—
Air-12	790	93	13	718	85	2	820	95	13	730	87	2	—
Air-14	785	93	14	778	91	3	830	96	14	770	91	3	+
Air-15	843	99	15	770	87	4	845	98	15	715	82	4	++
Air-16	793	96	16	743	83	5	855	98	16	642	66	5	+++
Air-18	788	91	17	570	56	6	860	99	17	530	49	6	++
Air-20	795	92	18	—	—	—	842	96	18	—	—	—	++
Sealed-12	753	87	19	793	94	8	792	93	19	778	93	8	—
Sealed-14	778	90	20	765	83	9	848	98	20	822	96	9	—
Sealed-15	800	95	21	805	84	10	865	101	21	840	97	10	+
Sealed-16	813	91	22	720	67	11	885	100	22	595	57	11	++
Sealed-18	775	88	23	540	40	12	852	97	23	438	32	12	++
Sealed-20	725	76	24	—	—	—	815	92	24	—	—	—	++

number of the stored samples gave better results than the check samples, especially those stored in the cold for six months. The following six samples showed distinctly lower values than the checks:

3 MONTHS	6 MONTHS
L-18-air	L-16-air
L-18-sealed	L-18-air
	L-16-sealed
	L-18-sealed

The wheats of all of these were dead as shown in Table VI. The following dead samples gave baking results almost equal to the checks:

3 MONTHS	6 MONTHS
L-16-air	L-15-air
L-18-sealed	L-15-sealed

These were next to the samples which had some or good viability. Thus if a sample is dead it may be only slightly or may be severely damaged. Good viability is a sign of no serious damage.

The dough from four of the laboratory-stored samples which had a lower baking value than the checks had a distinct foreign odor. The intensity of the odor is designated by the number of + signs. This odor was also noticed in three samples which had baking figures equal to the checks. Thus a dough which has a slight odor due to damage in the wheat may still give good baking performance. The odor in the dough compares with the viability test as among the most sensitive tests for damage.

A few specific gravity determinations made on samples differing widely in storage conditions did not show any correlation with the various treatments these samples had received. One reason for this may be that the wheat used in this experiment had undergone considerable weathering during harvest.

Curves on the recording dough mixer.—Curves on the recording dough mixer were made on a selected number of flours from the six-months storage lot. Several of these flours were extracted with petroleum ether for six days. After removal from the extractor, they were exposed to allow the ether to evaporate. Representative curves from both extracted, unextracted, cold, and laboratory storage are given in Figure 5.

Curve characteristics are due to two sets of factors: (1) those which are inherent in the gluten structure and (2) those which surround the gluten material. The former determines types of curves such as obtained from Tenmarq and Chiefkan. The second set of factors includes added salts, acids, or certain fatty materials. Fatty substances are most easily altered under unfavorable storage conditions. The purpose of extracting flours with ether was to ascertain whether the removal of the ether-extractable material would have any effects on the curve characteristics.

The two upper rows are from the samples stored in the cold and all the rest are from the laboratory-stored samples. No great amount of change was produced in the curve characteristics of those stored in the cold even with 18% and 20% moisture, nor were those from the ether-extracted notably different from the unextracted. Thus these curves corroborate the other tests, showing that no damage, or very little, had occurred in the samples stored in the cold.

The curves from the laboratory samples tell a different story. Curves 11 and 12 from the samples with 15% moisture show distinctly the beginning of those changes which were much more manifest when the moisture content was 16%; note curves 13, 14, 15, and 16. The extraction with ether had no effect that might not be due to variation in absorption. The amount of damage was still greater when the moisture was 18% as shown in curves 17, 18, 19, and 20. When the damage is very great as in this case the dough behaves more like a plastic mass, as shown in curves 17 and 18, which show no rise in the normal length of the curves. If the time of mixing is continued long enough, there will be a slow rise as shown in curves 19 and 20.

The curves presented show that the type of damage which had occurred in these samples is revealed by curve characteristics as well as by the baking tests.
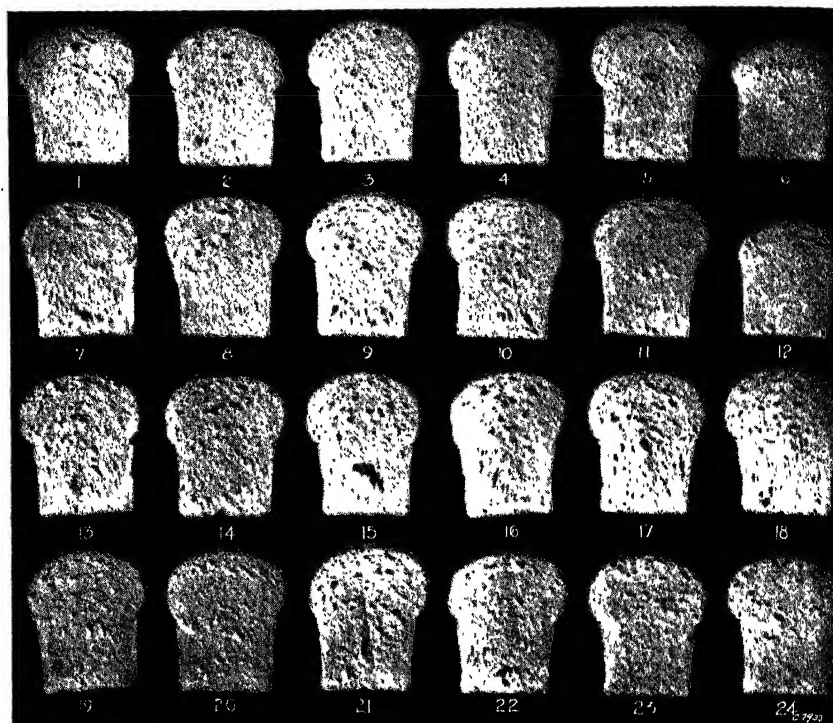
Fig. 3.   Photographs of loaves from the three-months storage samples.

Loaf No. on photograph	Sample No.	Baking No.	Loaf No. on photograph	Sample No.	Baking No.
1	L-3	19	13	C-12-A	16-1
2	L-12-A	3	14	C-14-A	17-1
3	L-14-A	4	15	C-15-A	18-1
4	L-15-A	5	16	C-16-A	20
5	L-16-A	6	17	C-18-A	1
6	L-18-A	7	18	C-20-A	2
7	C-3	15-1	19	C-12-S	8
8	L-12-S	14	20	C-14-S	9
9	L-14-S	15	21	C-15-S	10
10	L-15-S	16	22	C-16-S	11
11	L-16-S	17	23	C-18-S	12
12	L-18-S	18	24	C-20-S	13
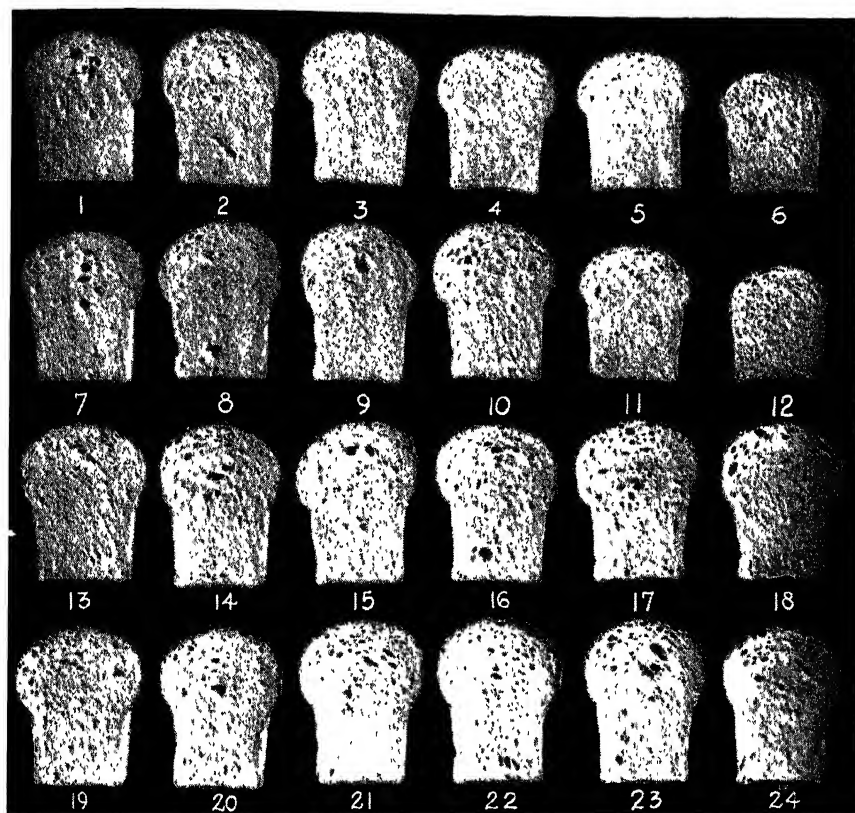
Fig. 4. Photographs of loaves from the six-months storage samples.

Loaf No. on photograph	Sample No.	Loaf No. on photograph	Sample No.
1	L-6 check	13	C-12-A
2	L-12-A	14	C-14-A
3	L-14-A	15	C-15-A
4	L-15-A	16	C-16-A
5	L-16-A	17	C-18-A
6	L-18-A	18	C-20-A
7	C-6 check	19	C-12-S
8	L-12-S	20	C-14-S
9	L-14-S	21	C-15-S
10	L-15-S	22	C-16-S
11	L-16-S	23	C-18-S
12	L-18-S	24	C-20-S
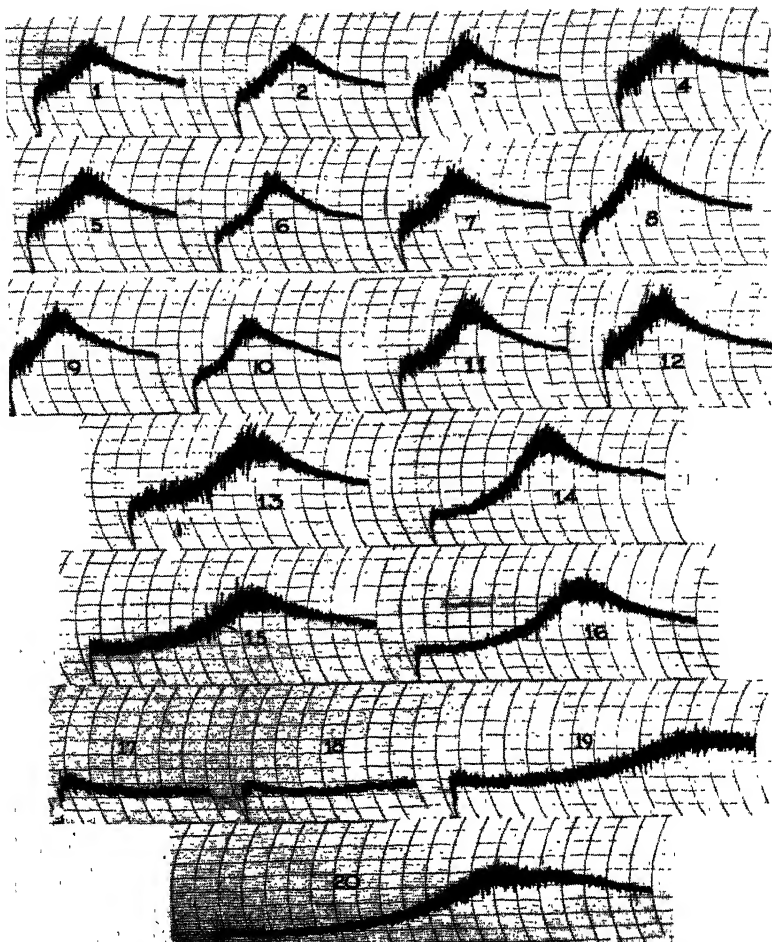
Fig. 5. Dough-mixer curves on the cold and the laboratory-stored samples.

Cold storage

(1) C—check 63%	(2) C—check extr. 75%	(3) C—18 air 64%	(4) C—18 sealed 65%
(5) C—20 air 61%	(6) C—20 air extr. 69%	(7) C—20 sealed 63%	(8) C—2 sealed extr. 68%

Laboratory storage

(9) L—check 63%	(10) L—check extr. 71%	(11) L—15 air 64%	(12) L—15 sealed 63%
	(13) L—16 air 62%	(14) L—16 air extr. 72%	
	(15) L—16 sealed 62%	(16) L—16 sealed extr. 68%	
(17) L—18 air 59%	(18) L—18 air extr. 67%	(19) L—18 air sealed 60%	
	(20) L—18 sealed extr. 68%		

Summary

Two years' work on storing wheat samples at various moisture contents in the laboratory and at 41°F. have been presented. Variations in air supply and duration of time were introduced the second year. The main tests used to measure the effects of these conditions during storage were: viability, test weight, soundness of grain, milling and baking tests, and curves made on the recording dough mixer.

There was no decrease in viability in the samples stored in the cold, while all those stored at higher moistures in the laboratory were dead. Wheat of good viability shows no damage to its milling and baking qualities. Unsoundness, as indicated by mustiness, developed at lower moistures in the laboratory than in the cold storage. In the latter, sourness developed in the samples with the higher moistures. This kind of damage was not reflected in the results of the baking tests. In fact these tests were shown to be much less capable of detecting damage than the viability and the odor tests. The altered curve characteristics obtained on the damaged samples were not due to changes in the ether-extractable substances but were due to alteration in gluten characteristics.

Wheat may be stored at 41°F. at high moisture for many months without suffering any damage to its milling and baking qualities. When wheat has good viability it has suffered no damage to the gluten structure even when a slight odor indicating unsoundness can be detected.

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AMERICAN WHEAT TYPES AND VARIETIES AS DISTINGUISHED BY FARINOGRAMS AND EXTENSOGRAMS

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I. Testing Soft Wheat Flours

The application of practical baking tests to the testing of soft wheat flours has not proved to be entirely satisfactory. These flours are used for a diversity of purposes in the baking of biscuits, cakes, pastries, and crackers. Accordingly the development of a single baking test that will disclose the adaptability of any flour to several of these purposes is not feasible. Moreover, it is difficult to simulate in the laboratory the conditions to which the flour will be subjected in certain commercial baking operations.

Chemical analyses and such physical tests as the viscosity of acidulated flour suspensions have proved of service in the soft wheat flour industries. None of these have proved wholly adequate, however. The farinograph served to advance such testing procedures and has afforded one means for classifying wheat types and varieties to a degree that was not possible prior to its application to such studies.

Thus, as indicated by the six farinograms in Figure 1, there are sharp differences discernible in the behavior of such flours when thus tested. Various samples of soft red winter wheat produced in Illinois differed widely, as disclosed by farinograms *A* and *B* at the top of the figure. Sample *A* is typical of a weak flour of this class, while sample *B* is distinctly on the strong side. Both farinograms are different from that which resulted from testing the sample of Federation. The latter is typical of the flours milled from this variety, and less variability was encountered among the several samples of this, and of the White Club and Soft White types. This facilitates the identification of individual samples in such instances. Thus the White Club farinogram discloses very rapid dough development to the maximum consistency, together with a wide curve which does not diminish substantially in width, but decreases steadily in consistency with extended mixing. This is the farinogram of a flour adapted particularly to pastry and cookie baking.

Federation wheat flour yielded a farinogram which also disclosed a rapid dough development, followed by an interval during which the consistency changed very little, although the curve width decreased appreciably. Thereafter a substantial decrease in consistency was regis-

tered. In general the curve width was narrower than the White Club, and the general outline of the curve was markedly different from the latter. Absorption to yield a unit maximum consistency was 2%–3% greater in the Federation than in the White Club flour sample.

Soft white wheat (Michigan) flour was intermediate between the Federation and White Club in many respects, although the curve width
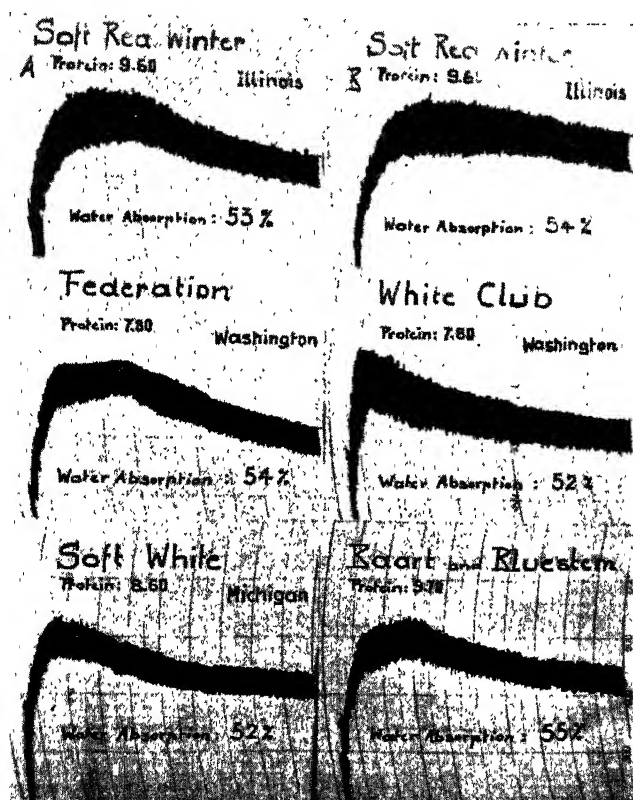
Fig. 1. Farinograms of prominent American soft wheat varieties and classes.

was narrower. Also the dough became quite sticky, which contributed to the irregularity of the farinogram. Absorption was in the same range as the White Club. Such flour is adapted to the baking of angel food cake.

Baart and Pacific Bluestem wheat flour yielded a farinogram disclosing a slower rate of dough development than the Federation, White Club, and Michigan Soft White wheat flours. In this respect it was similar

to the Soft Red winter wheat flours, but differed in having a narrower curve width. Water absorption was higher than with the other soft wheat flours. At the same level of protein content the Baart-Bluestem flour farinograms might be expected to resemble the Federation flour farinograms, but most of the Baart and Bluestem wheat of commerce tends toward an appreciably higher protein content, which distinguishes it from the Federation wheat.

Experience has indicated that certain wheat types and varieties yield such distinctive farinograms (when corrected to a known or constant protein level) that they can be distinguished by farinograph tests. The latter may even serve to indicate the kinds of wheat in a mixture and thus be of practical service in duplicating blends in competitive flour production. In interpreting farinograms consideration must be given to protein content, extraction in milling (which is reflected in part in
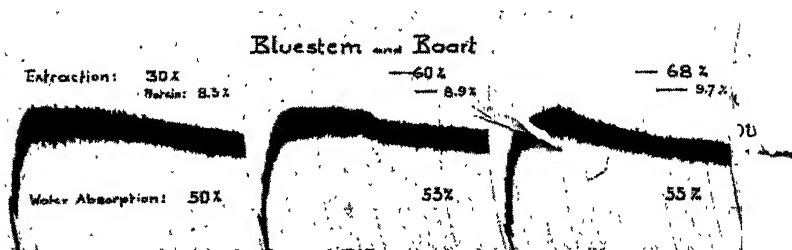
Fig 2.   Influence of percentage of flour extraction on farinogram.

the ash content), and granulation of the flours under test. The latter is of least consequence in terms of its direct effect upon the shape of the farinogram, but may be of major significance in practical cake baking.

Extraction in milling is of large consequence, however, as demonstrated by the three farinograms in Figure 2. These represent flours from the same mill mixture of Baart and Bluestem but milled to 30%, 60%, and 68% extraction [1] respectively. The farinograms differ as greatly as do farinograms of flours from different wheat varieties. It should be stressed here that the six flours represented by the farinograms in Figure 1 were all of about the same percentage extraction, namely 68%, and accordingly are directly comparable so far as flour characteristics are concerned.

Effect of Certain Flour Treatments

Certain flour treatments, notably with oxidizing agents, are not disclosed by the farinograph, however, and in order to adequately study

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[1] Extraction is recorded here in the European manner, namely, in terms of the percentage of the original wheat, rather than in terms of the percentage of total flour.

their effect upon dough properties, recourse should be had to the extensograph. A significant indication of the usefulness of the extensograph in testing soft wheat or pastry flour doughs is indicated by the graphic records of Figures 3 and 4. In Figure 3 appear the extensograms [2] of
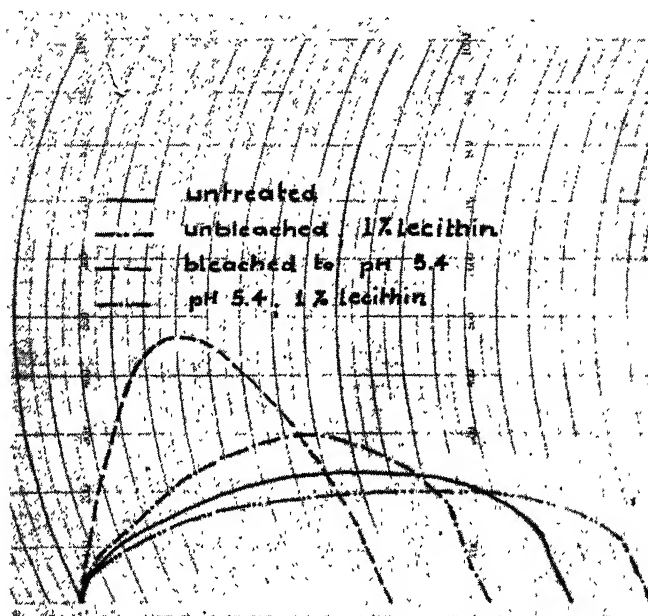
Fig. 3. Extensogram data showing the results of the treatments indicated.

Fig. 4. Photographs of cookie baking tests involving the treatments indicated.

doughs made from a single flour treated in different ways, and compared with the untreated flour. The treatments included (1) addition of 1% of lecithin, (2) bleaching with Cl_2 to pH 5.4, and (3) a combination of lecithin and bleaching.

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[2] For a discussion of extensograms see the first paper in this series, " Prediction of baking value from measurements of plasticity and extensibility of dough—I," Cereal Chemistry 17: 78–100, January 1940.
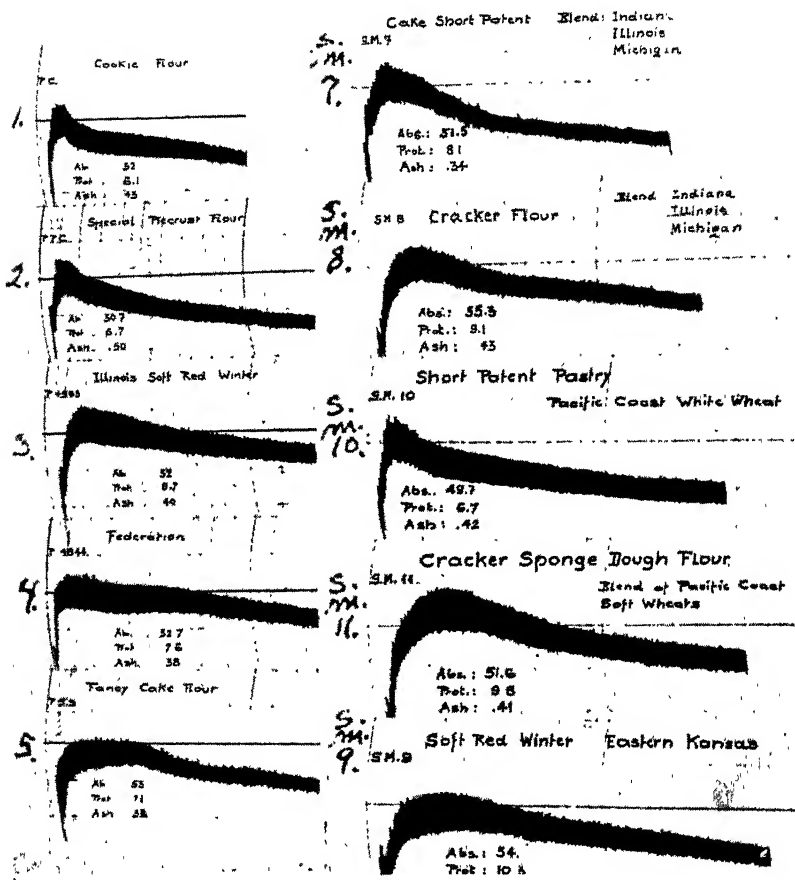
Fig. 5. Farinograms of several types of soft wheat flour.

Fig. 6. Farinograms of cake, cracker and pastry flours.

Superimposing lecithin upon the untreated flour resulted in increasing the extensibility of the resulting dough, as evidenced by the increase of the extensogram along its horizontal or E axis. Bleaching to pH 5.4 with Cl_2 had the reverse effect, decreasing the extensibility E, sharply increasing the force F to extend the dough as recorded on the vertical axis, and thus substantially altering the F/E ratio. When lecithin was then superimposed upon the chlorine-bleached flour dough, there was a tendency toward regaining the characteristics of the untreated flour dough, although the E value of the latter was still greater and its F value significantly less than that of the dough with the combined lecithin and Cl_2 treatment.

Figure 4 shows photographs of cross sections of cookies baked from the four doughs which are represented in Figure 3. The round disks of dough from which the cookies were baked were uniform in the raw state. The effect of lecithin upon the " spread " of the cookie doughs is evident from the increased diameter. Chlorine bleaching (3rd row) evidently " tightened up " the dough and reduced the " spread " or flow in baking considerably. Again, the combination of Cl_2 and lecithin treatments (4th row) tended to restore the properties of the untreated flour dough. The correlation between the extensograms and the cookie baking tests is striking.

Classifying Soft Wheat Flours for Special Purposes

Differences between individual flours milled from soft wheats are demonstrated by the farinograms and extensograms represented in Figures 5, 6, 7, 8, and 9. Thus the farinogram numbered 5 (Fig. 5) and 7 (Fig. 6), representing flours used for cake baking, were milled from
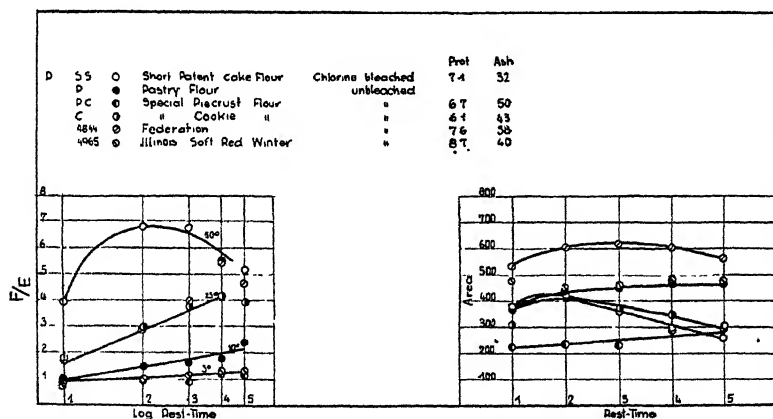
Fig. 7. Extensogram data of the flours for which farinograms are shown in Figure 5.

different types of wheat than the flours yielding farinograms numbered 1 (Fig. 5) and 10 (Fig. 6), which last two are typical cookie and pastry flours. These latter resemble the White Club flours, while 5 and 7 appear to have been blends of soft white and soft red winter wheat flours.

Cracker dough flour numbered 8 (Fig. 6) yielded a farinogram of the same general characteristics as the soft red winter wheat flours shown in Figure 1. The cracker sponge flour farinogram (No. 11) is somewhat wider than the cracker dough flour (No. 8). Extensograms of these two flours are quite different (Fig. 8), occupying the upper posi-

tion (No. 8) and the lowest position (No. 11) in the F/E ratios shown at the lower left of the figure.

This discloses that the cracker dough flour resisted extension, *i.e.* had a relatively high F value, but exhibited little extension, *i.e.* the E value was low, and hence the F/E ratio was high after several hours of rest time had been allowed to the dough under test. Since the time of fermentation in the sponge stage is much longer than in the dough stage in cracker manufacture, it might well follow that doughs made from both of these flours would yield similar extensograms if fermented for the widely different periods of time accorded them under commercial conditions.
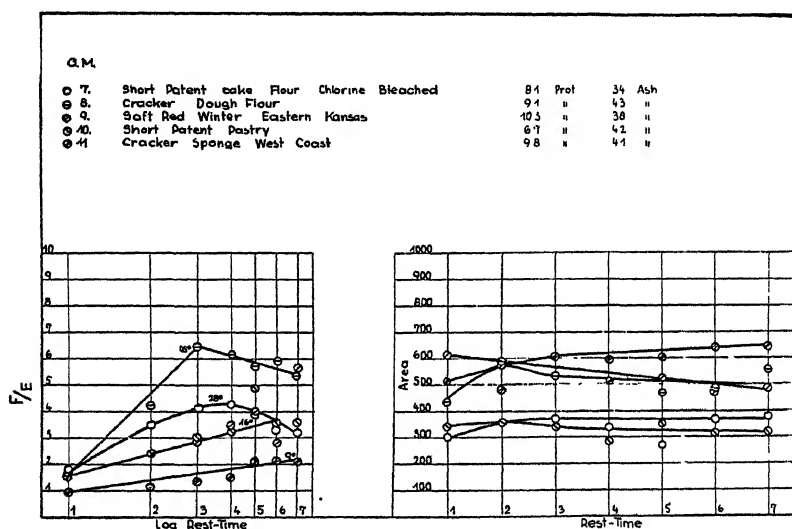
Fig. 8. Extensogram data of the flours for which farinograms are shown in Figure 6.

The two pastry and cookie flours, Nos. 1 (Fig. 5) and 10 (Fig. 6) yielded farinograms of the same general character, and it has already been suggested that these indicate White Club wheat as the principal constituent of the mill mixture. Their extensograms (Figs. 7 and 8) also have some features in common, both in F/E ratios and area, and exhibited only a small or moderate change in the F/E ratio with an increasing rest time. The special pie crust flour No. 2 (Fig. 5), designated " P.C." in Figure 7, also yielded a farinogram indicating that it might have been milled from White Club wheat. Its low protein content is probably reflected in the small area under the extensogram, recorded graphically at the right in Figure 7. The lack of change in the F/E
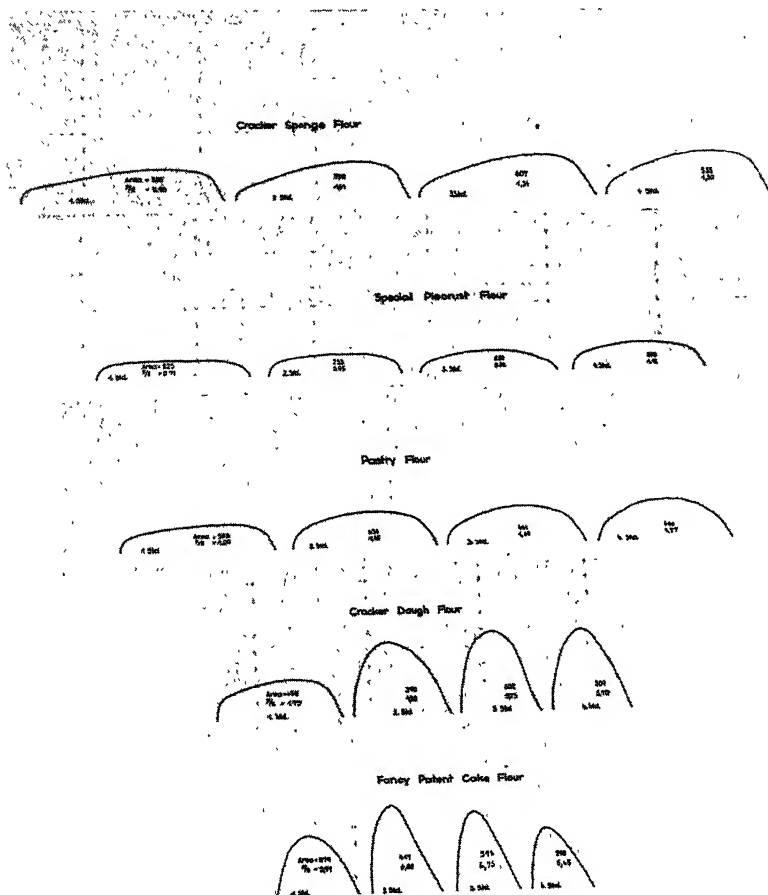
Fig. 9. Actual extensograms for which the data are recorded in Figures 7 and 8.
("Std." is the abbreviation of "Stunde," the German word for *hour*.)

ratio with increasing rest time may be the consequence, in part, of its relatively high ash content.

The actual extensograms of five of the flours from which certain of the constants recorded in Figures 7 and 8 were computed, are shown in Figure 9. The F/E ratio of the "special pie crust flour" is obviously very low, and changes little between the first hour, labeled "1 Std.", the abbreviation of *Stunde* (German for hour), and the fourth hour of rest after mixing. The "pastry flour" ranked second in these particulars, followed by the cracker sponge and cracker dough flours in the order named. The "fancy patent cake flour" was quite distinc-

tive, with a relatively high F/E ratio, passing through a maximum after standing two hours and then receding somewhat. The area under the extensograms is not large, however, and it is probable that this combination of properties may be a reflection of the short extraction of this flour and the bleaching·treatment accorded it.

In Table I we present a consolidated summary of the characteristics of several types of flour, based upon certain broad, general opinions of the American baking industry. It is recognized that limits cannot be expressed too definitely, however, since bakers are not uniformly agreed as to what represents an ideal flour for every specific purpose.

TABLE I

GENERAL CHARACTERISTICS OF FLOURS DESIGNED FOR CERTAIN SPECIAL PURPOSES

Characteristic	Short patent cake flour [1]	Cookie flour	Cracker flours	
			Dough	Sponge
Extensograph area	300–450	350–500	500–600	600–700
Specific form [2]	>3½	2½–3½	4–6	1–2
Crude protein, %	—	6.5–7.5	—	—
Ash, %	0.32–0.34	0.38–0.43	0.40–0.44	0.40–0.44
Granulation	Very fine	Coarse	Medium	—
pH	5.3–4.8(Cl₂)	—	—	—
Water absorption [3] (15% basis)	—	49–52	·54–57	—

[1] Especially for angel food and layer cake.
[2] Specific form is the F/E ratio after 2¼ hour rest period.
[3] Consistency level 500 Brabender farinograph units. Farinogram of short patent cake flour should range between S.M. 7 in Figure 6, and No. 5 in Figure 5. Farinogram of a cookie flour should resemble No. 1 in Figure 5, reaching maximum consistency early, and then decreasing rapidly in consistency thereafter, but without a major decrease in the curve width. Farinogram of a cracker dough flour should conform to curve marked S.M. 8 in Figure 6. Farinogram of a cracker sponge flour should indicate somewhat greater strength than that of the cracker dough flour.

II. Testing Hard Wheat Flours

The laboratory baking test has been more useful in testing hard wheat flours than soft wheat flours, but still it does not give all the desired information. The blending value of a flour is inadequately indicated by this test. The baker's judgment of the dough behavior during fermentation cannot be recorded with satisfaction. Of course it is possible to record the volume and appearance of the finished loaf, but deductions from the latter concerning dough properties or blending possibilities of a flour are often vague and unreliable. Thus it is shown in Table II. that volume, score, and fermentation time can be nearly the same with flours of equal protein content but of decidedly different dough properties. The different wheat varieties compared are known to have quite different dough properties but still their loaf volumes and bread scores compare within the experimental error. Whereas the baking technologist who carried out these tests could distinguish the widely different

flours from their appearance in the dough stage, such discrimination was not possible with the loaves baked from these flours. Since the quality of a flour is not only determined by the finished loaf but equally well by the behavior of the dough during the various dough manipulations it follows that the customary baking test is at times inadequate.

This explains why the present trend of cereal research is towards physical methods of dough testing. In two earlier papers (footnotes 2 and 3) some basic principles of dough reactions such as are revealed by

TABLE II

DATA ON FLOURS HAVING DIFFERING DOUGH PROPERTIES

Grade	Class of wheat	State where grown	Crude protein, % in 60% extraction flour	Volume (cc.)			Score		
				Fermentation time in hours					
				3	4	5	3	4	5
1 DK	Hard Red Spring	N. D.	13.1	2530	2475	2475	92	90	89
1 DHW	Dark Hard Winter	Kan.	12.3	2515	2490	2475	92	90	89
1 HWh	Hard White	N. D.	12.8	2460	2475	2420	91	90	87
1 DHW	Dark Hard Winter	Okla.	12.4	2430	2430	2420	90	89	88
2 D° Sp.	Hard Red Spring	Ill.	13.1	2585	2515	2460	93	91	88
1 HS	Hard Spring	—	13.7	2490	2475	2475	91	91	90
2 HWh	Hard White	N. D.	12.8	2530	2475	2475	90	91	90
Average			12.9	2505	2476	2457	91	90.3	89
2 HWh	Hard White	—	11.8	2430	2460	2375	89½	89½	87½
2 DK° Sp.	Dark Spring	Ill.	11.5	2475	2475	2445	90	88½	87
3 HW	Hard Winter	Neb.	11.7	2405	2375	2360	90	88	88½
2 DHW	Dark Hard Winter	Mont.	11.7	2445	2390	2360	91	88½	87
HRW	Hard Red Winter	Ill.	11.0	2460	2475	2390	93	90½	87½
HW	Hard Winter	Kan.	11.2	2430	2415	2390	90	89	87½
1 DK	Dark Spring	Ill.	11.7	2460	2430	2430	90½	88	87
2 HW	Hard Winter	Minn.	11.7	2445	2460	2330	91½	90	88½
2 X	Not classified	Kan.	11.3	2460	2445	2415	90	90½	87
1 D° Sp.	Dark Spring	Ill.	11.4	2460	2445	2360	91	90½	87
1 HW	Hard Winter	Neb.	11.2	2475	2445	2430	90½	90	88
Average			11.5	2449	2437	2380	90.7	89.2	87.5

the farinograph and extensograph were discussed. The flours used for the experiments were mostly of commercial origin. The following section discusses data derived from testing semicommercially milled flour from special wheat samples obtained from various flour mills, and from pure wheat varieties obtained from the Northwest Crop Improvement Association. In case of the wheat variety samples the general baking behavior had been indicated in broad terms by the mills, while the general rating as regards baking quality of the pure wheat samples could be taken from a report of the Northwest Crop Improvement Association, which consolidates and summarizes the results of individual collaborative baking tests.
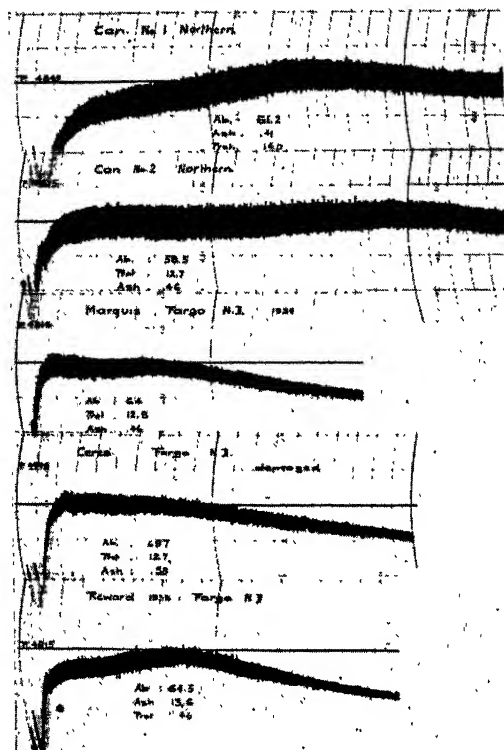
Fig. 10.   Farinograms of the first series of hard spring wheat flour samples.
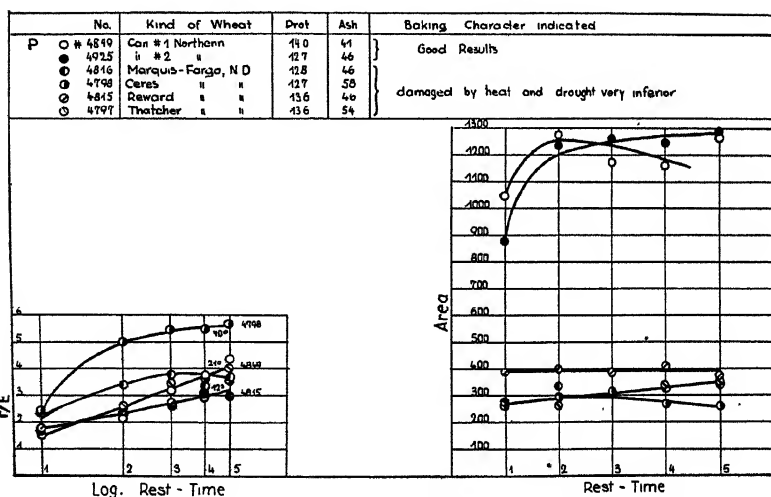
Fig. 11.   Extensogram data of the flours for which the farinograms are shown in Figure 10.

In the rating of flours according to extensogram data primary consideration was given to curve surface; however, in instances of similar surfaces preference was given to flours which yielded doughs of larger extensibilities.
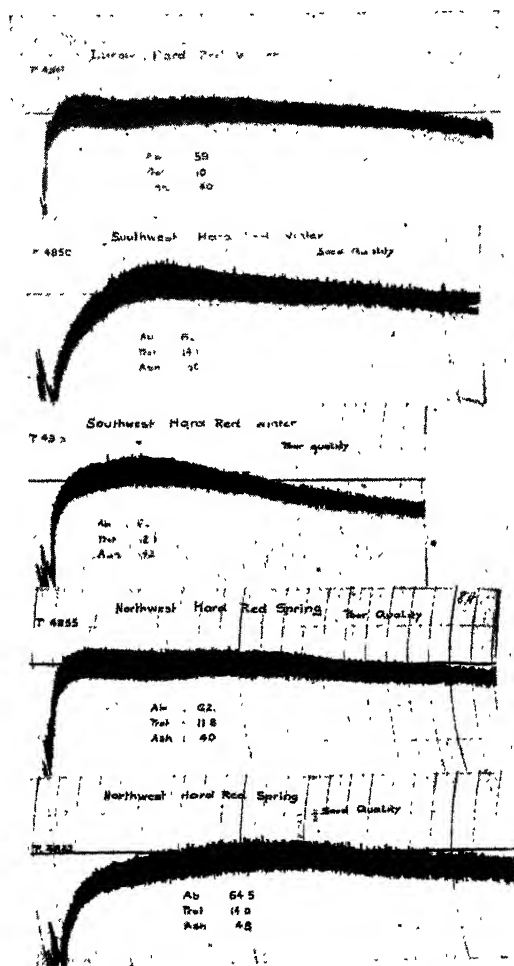
Fig. 12. Farinograms of the second series of hard wheat flours.

Certain relationships between farinograms and extensograms of hard wheat flours become apparent on comparing the data recorded in Figures 10 and 11. In Figure 10 the third, fourth, and fifth farinograms suggest flours milled from wheats that have been damaged by weather conditions such as drought. These faults are evidently reflected in the area

under the extensograms recorded at the lower right in Figure 11. On the other hand, the " strong " farinograms of the two Canadian flours are supported by the large area under their extensograms. Moreover the extensograms suggest a substantial positive response to bromate treatment, since their oxynumbers $\left(\dfrac{\text{Area}}{F/E \times 10}\right)$ of 51 and 42 respectively are in the positive range indicated by Munz and Brabender.[3] Canadian flours of the type represented by these flours are known to exhibit positive responses to bromate treatment.

P	No	Kind of Wheat				Ash	Prot	Baking Character indicated
o	4832	Northwest Hard Red Spring			untreated	48	14.0	Good Results
●	4855	" " " "			"	45	11 8	Poor "
◑	4850	Southwest " "		Winter	"	.36	14 1	Good "
◔	4913	" " "		"	"	.42	12 1	Poor "
◕	4903	Illinois " "		"	"	.40	10 0	Fair "
◒	4832	Northwest " "		Spring	003% Bromate	.48	14 0	

4832 + Bromate 22°
4913 18°
4855, 4850 14°
4903 10°
4832 .7°

Log Rest - Time

Rest Time
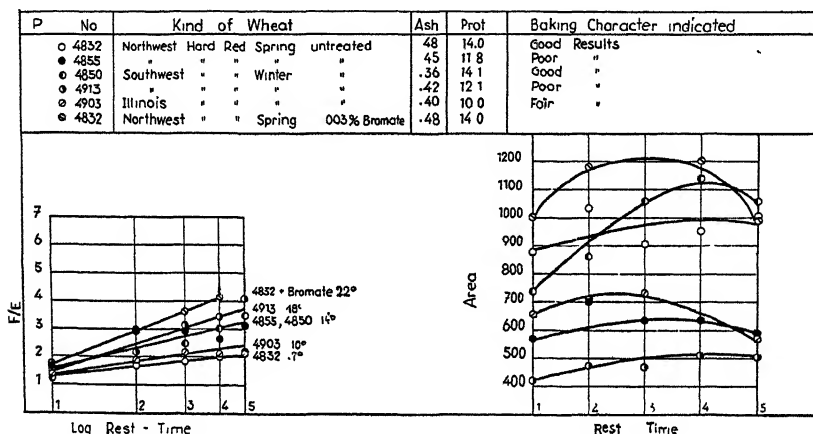
Fig. 13. Extensogram data of the flours for which the farinograms are shown in Figure 12, together with ash and protein content, and the baking characteristics disclosed by the collaborative tests of the Northwest Crop Improvement Association.

A second series of five hard wheat flours were subjected to study, and the resulting farinograms are shown in Figure 12. The first of these hard red winter flours (upper farinogram) is representative of a type possessed of only fair baking quality. The second in order is of good quality, while the third is poor. In fourth position is a poor quality of hard spring wheat flour. This, and the first or upper farinograph, are typical of flours possessed of " short " gluten. The curves show a rapid dough development which reaches maximum consistency and curve width in about half the time that is required in mixing such strong flours as are represented by the second and the fifth farinograms.

Extensograms of the same five flours were correlated with the farinograms as shown by their analyses recorded graphically in Figure 13. The two superior flours mentioned above (Nos. 4832, spring wheat, and 4850, winter wheat) yielded extensograms with an area of 1000 to 1200.

[3] Emil Munz and C. W. Brabender: Extensograms as a basis of predicting baking quality and reaction to oxidizing agents, Cereal Chem. 17: 313–332, May 1940.
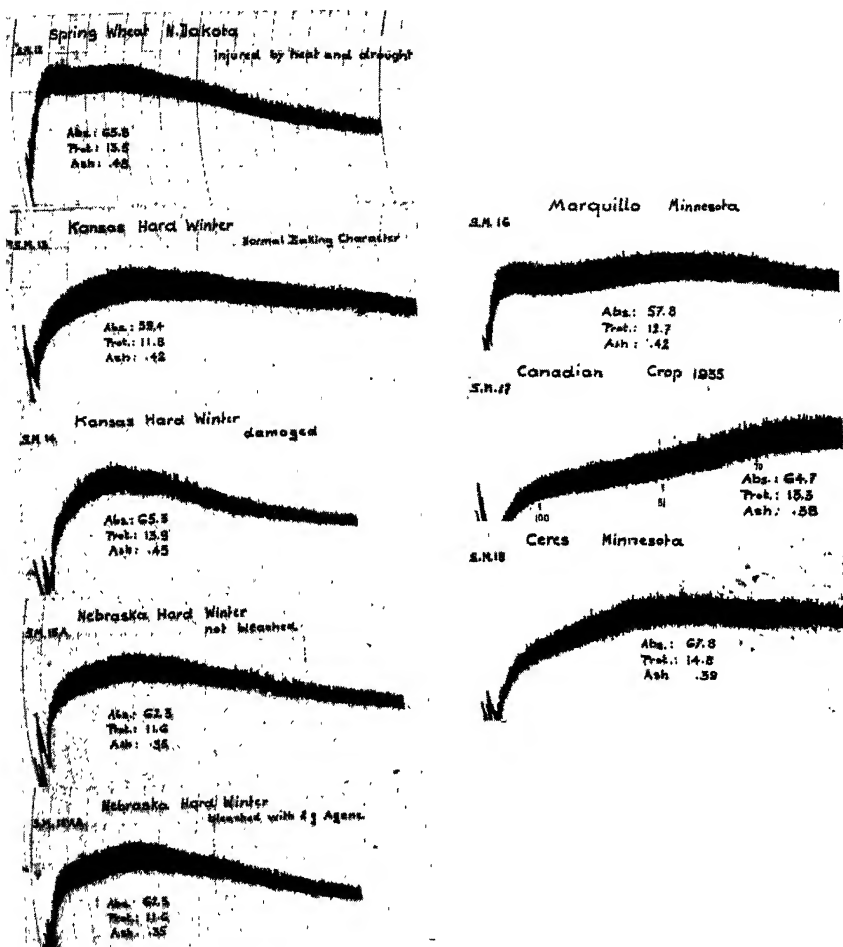
Fig. 14. Farinograms of the third series of hard wheat flours.

The oxynumber of the winter wheat flour was 45, that of the spring wheat flour 28, which is in keeping with their known reaction to oxidizing agents.

A third series of eight flours was then tested, and the farinograms are shown in Figures 14a and 14b. From these curves the spring wheat flours would be rated in the following order of baking quality, Nos. 17, 18, 16, and 12. The last named behaved as though it was milled from wheat that had been injured by heat and drought before harvest. The area under its extensograms (Fig. 15) was also low, and in the range of
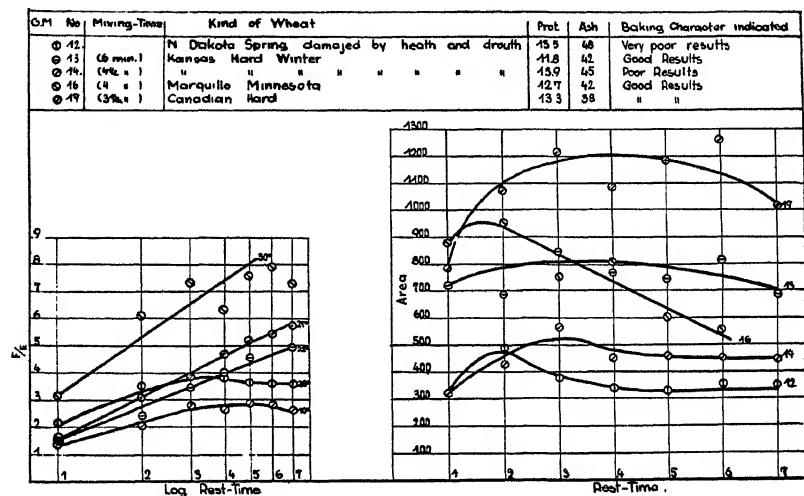
Fig. 15.   Extensogram data and baking characteristics of the flours for which the farinograms are shown in Figure 14.
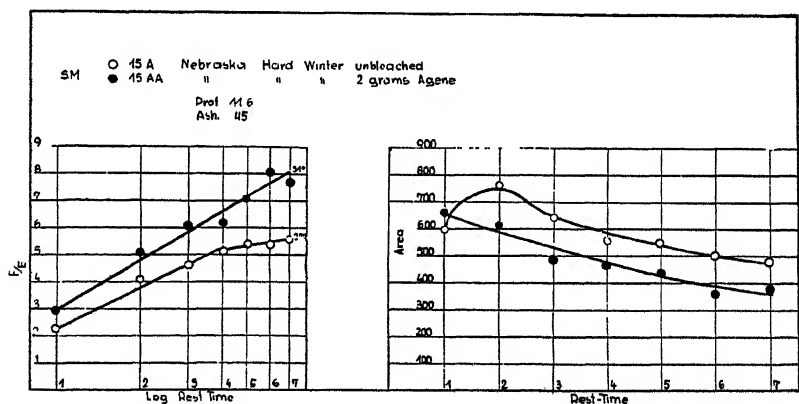
Fig. 16.   Extensogram data of a Nebraska hard winter wheat flour, untreated, and treated with Agene.

470 at the maximum.   The Marquillo (G.M. 16) farinogram suggested a short gluten, and this was supported by the graph of its extensogram areas with progressing rest time shown at the right in Figure 15.   After the second hour of rest, the area decreased sharply with the lapse of time.   It likewise had a low oxynumber, 13, which further demonstrated its " short " character.

The superior flour of this series was No. 17, with an extensogram area over 1200, and decidedly above the other spring wheat samples.

Kind of Wheat			Prot.	Ash	Loaf Vol	General Rating	Rating according to Curve Surface
Crop 1935							
○ 3640 Marquis	Havre	Mont	144	34	98	4	3
● 3638 Ceres	"	"	147	35	98	2	2
◓ 3637 Reward	"	"	145	34	104	3	4
○ 3639 Thatcher	"	"	147	33	102	1	1
⊘ 3643 Comet	"	"	130	36	94	5	5
◉ 3648 Reward	Leeds	N.D	135	3	106	1	1
● 3642 Thatcher	"	"	132	40	96	2	2

Log Rest Time   Rest - Time
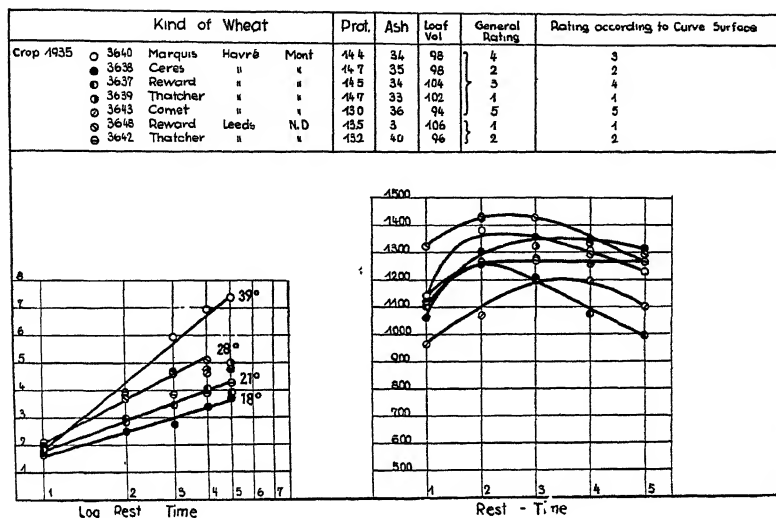
Fig. 17. Extensogram data and results of baking tests of the series of flours subjected to test by the Northwest Crop Improvement Association.

Of the two Kansas flours, G.M. 13 and 14, the latter appeared from the farinograms to have been milled from damaged wheat. That No. 14 was inferior to No. 13 was further evident from the area under its extensogram which was about 300 units less than the latter.

Action of Agene bleaching treatment on the Nebraska winter wheat flour No. 15 was significant. The farinograms of the untreated and treated flours, shown in Figure 14a, were not greatly dissimilar, but the data from the extensograms in Figure 16 were very different. To begin with, this flour had been milled at least eight months before the Agene treatment was applied. Its oxynumber at that time was only 17, which constituted a basis for predicting a negative response. This prediction was supported by the extensograms, which showed a higher F/E ratio, and a smaller area for the treated flour, after the doughs had rested for several hours. Obviously the extensograms were more useful in testing for response to such treatments than the farinograms.

A series of flours produced in a semi-commercial experimental mill from several wheat varieties grown in the northwestern United States became available for tests. These flours had been subjected to extensive collaborative baking tests under the supervision of the Northwest Crop Improvement Association, with the average results shown in the tabulation at the top of Figure 17. While the farinograms of these seven flours were of interest, they constitute a less satisfactory indication of baking quality than the extensograms, and are omitted from this paper.
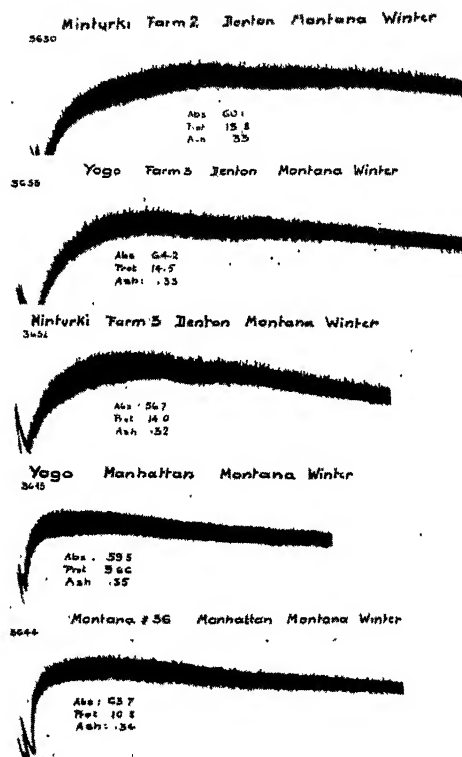
Fig. 18.   Farinograms of five Montana winter wheat flours.
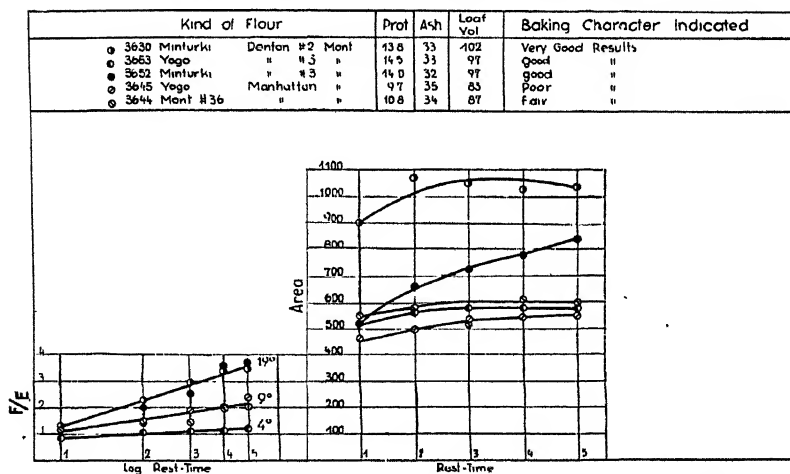
Fig. 19.   Extensogram data and baking characteristics of the Montana hard winter wheat flours for which the farinograms are shown in Figure 18.
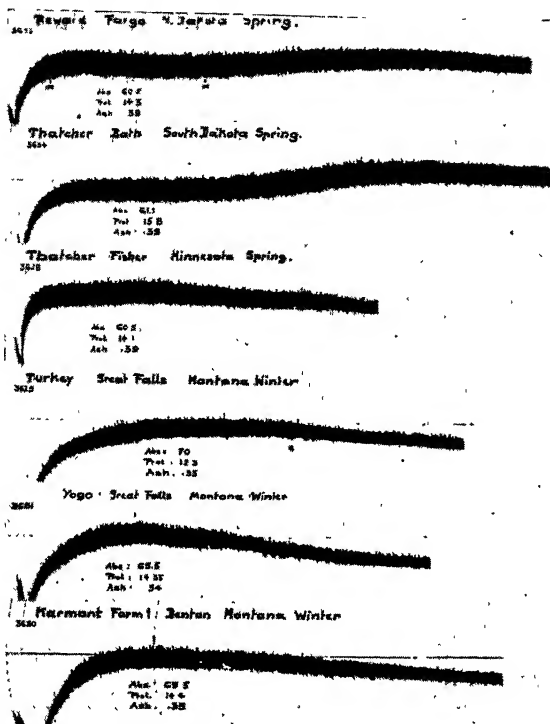
Fig. 20. Farinograms of the sixth series of hard wheat flour samples.

Crop 1935		Kind of Wheat		Prot.	Ash	Loaf Vol	General Rating	Extensograph Rating (Area)
O 3632	Reward	Fargo	N D	14.3	.39	102	Very good	Very large
● 3634	Thatcher	Bath	S.D	15.8	39	107	" "	" "
◐ 3628	"	Fisher	Minn	14.1	39	101	good	large
◔ 3629	Turkey Red	Great Falls Mont		13.0	35	86	Fair	medium to small
◒ 3651	Yogo	" " "		13.3	.34	95	"	" " "
◍ 3650	Karmont	Benton #4 Mont		14.4	35	90	"	small
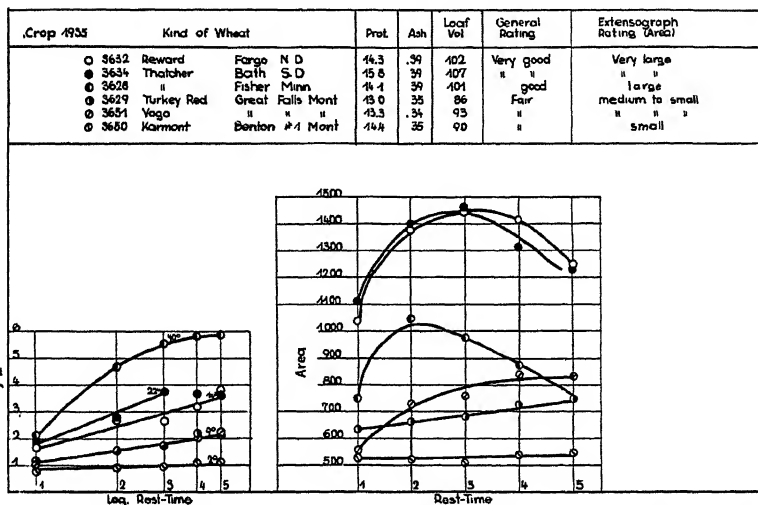
Fig. 21. Extensogram data and general rating of baking quality of the six flours for which the farinograms are shown in Figure 20.

The rating according to the curve surface of the extensograms shown in the right-hand column of the tabulation in Figure 17 correlates closely with the average general rating assigned by the numerous baking technologists who passed judgment upon this series of flours. Unfortunately the available summary of the baking tests does not afford a basis for correlating fermentation tolerance as disclosed by actual tests, with the change in areas under the extensograms as a function of rest time.
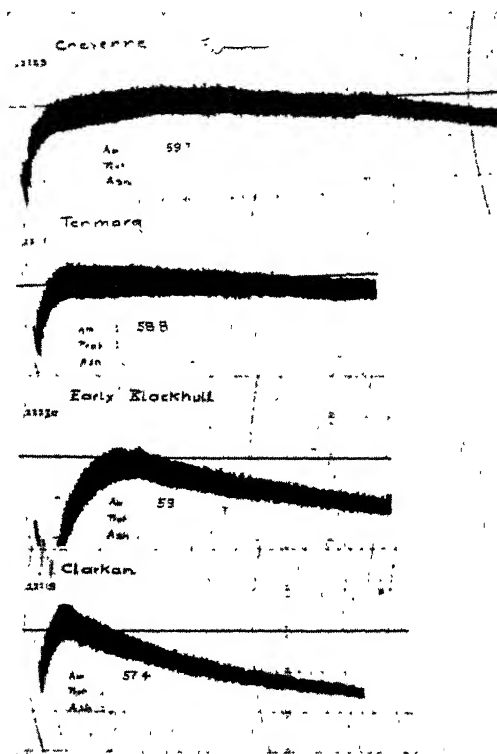
Fig. 22.   Farinograms of four Kansas winter wheat varieties.

Five Montana winter wheat flours were used in the next phase of these researches. These had previously been subjected to baking tests with the general results indicated in the last column of the tabulations at the top of Figure 19. Their farinograms are shown in Figure 18, and from them one would predict that Minturki No. 3630 would give the best results in a baking test, while Yogo 3645 would be the poorest of the five. These flours occupied those positions in the baking trials, and, moreover, that is also their relative position in terms of area under the

extensograms recorded in Figure 19. In fact the extensogram data serve again to arrange the five flours in the order of their rating in baking trials.

Still another series of wheat flours, including three milled from hard spring wheats and three milled from hard winter wheats, were subjected to comparative tests. From their farinograms, shown in Figure 20, the Thatcher grown at Fisher (No. 3628) would not be expected to perform as well as the Thatcher from Bath (No. 3634), or the Reward from
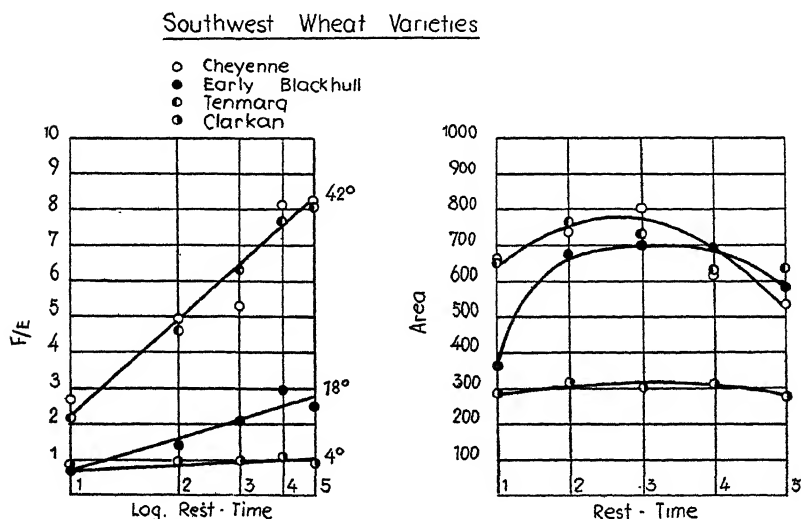
Fig. 23. Extensogram data of the four Kansas winter wheat varieties for which the farinograms are shown in Figure 22.

Fargo (No. 3632), and the cooperating baking technologists of the Northwest Crop Improvement Association rated them in that manner. Reference to the extensogram areas recorded in Figure 21 shows the flours Nos. 3632 and 3634 to be outstandingly high in their curve areas, and very similar to each other, with No. 3628 distinctly lower.

The three winter wheat flours in this series gave farinograms quite different from those of the spring wheat flours. Also the extensogram areas were lower, and the baking tests placed them in the same relation— namely, below the hard spring wheat flours.

Finally, a series of four Southwestern winter wheat flours were compared. It will be noted that the Clarkan sample was in a class by itself, as evidenced by the farinogram, and by the small area under its extensogram, which would serve to rate it as decidedly inferior to the hard wheat flours with which it is here compared.

Summary

Among the soft wheat types the combination of test data derived from farinograms and extensograms serves to classify them as to origin, *i.e.* the class or variety of wheat from which they are milled and as to their adaptability to special uses in baking.

Extensograms are proving singularly useful in disclosing the suitability of flours for cake, cookie, and cracker production. A basis of classification is proposed for use, with emphasis upon the specific form and area under the extensograms, together with other characteristics.

Superimposing lecithin upon untreated cookie flour increased the extensibility of dough made from the mixture, as evidenced by the increased E axis of the extensogram. Bleaching with Cl_2 to pH 5.4 had the reverse effect. The combination of chlorine treatment and lecithin restored the properties to approximately those of the untreated flour. Actual cookie baking tests confirmed the extensogram tests.

When hard wheat flours were subjected to tests with the farinograph and the extensograph, it proved possible to correlate the resulting data with the results of baking tests.

Further confirmation was afforded of the usefulness of extensograms in predicting the reaction of hard wheat flours to treatment with oxidizing agents, including Agene and bromate.

On the basis of the experimental evidence reported in the two preceding papers [4] and in this paper it seems that the following generalizations are justified as far as practical deductions from farinograph and extensograph data are concerned:

I. Flour characteristics that can be deduced from the farinogram:

> *A*. Water absorption capacity.
>
> *B*. General strength (European conception) from
> > 1. Point to optimum development.
> > 2. Rate of consistency decrease after point of optimum development.
> > 3. Curve width.
>
> *C*. Degree of gluten hardening (shortness).
> > Indicated by quick development to optimum consistency and optimum curve width. It may be caused by age, heat treatment or varietal influences.
>
> *D*. Mixing sensitivity: flours that are overmixed easily give
> > 1. Strong curves which show rapid development to optimum curve width and consistency.

[4] See footnotes 2 and 3.

  2. Strong curves with very slow development to optimum curve width, the curve width being rather small (bucky flours).

  3. Weak curves showing rapid decrease in consistency and curve width.

II. Flour characteristics deduced from extensogram are:

  A. Prediction of loaf volume, from area.

  B. Bromate response from oxy-number.

  C. Fermentation time, from time to reach optimum area.

  D. Fermentation tolerance may possibly be deduced from expressions involving

    1. Change in area on extended rest-time.

    2. Change in the ratio F/E as expressed by the angle of the line connecting the different F/E values with the horizontal time axes.

    3. Change in oxy-number on extended rest-time.

  E. Deviation from " potential dough properties."

  F. Molder tolerance.

THE COUNTING OF YEAST CELLS IN BREAD DOUGHS

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Many uncertainties exist regarding the behavior of yeast in a fermenting dough. Some baking technologists have maintained that yeast does not multiply in a dough, but merely produces leavening gases. Others assert that yeast proliferation takes place. A knowledge of the actual number of yeast cells in doughs when mixed and after various periods of fermentation is of considerable assistance in the scientific study of fermentation problems. The method presented in this communication was developed to study the effect of nutrient salts on yeast growth, but should be of value in the consideration of other problems of fermentation.

Lindet (1910) studied the multiplication of yeast in dough by a plating method. Such methods, however, present difficulties in that yeast cells tend to form clumps, and the counts determined by such techniques are low. Geere and Geere (1922) determined the growth of yeast in doughs by a microscopical method of counting the cells. Simpson (1936) reported a method for counting yeast cells in doughs which was based essentially on the method of Turley (1924). The

principle of this method is the dispersion of the dough with pepsin and hydrochloric acid, the digestion of the starch cells with diastase, the staining of the cells with methylene blue, and counting in the haemacytometer.

The method developed in this laboratory predated the publication of Turley, and has since proved of value over a considerable length of time.   It avoids the difficulties of the previously published techniques, and is considerably simpler.

Procedure

Take a 20-gram sample of dough, place in a 2-liter beaker, add 1460 ml. of distilled water, 5 ml. of chloroform, and 10 g. of sodium chloride.   The chloroform is added to prevent further growth of the yeast cells while the counts are being made, but, inasmuch as it does not mix with the solution, no account need be taken of it when considering the dilution.   The salt is added to bind and hold the gluten in a ball and thus prevent its disintegration during the washing process.

Wash the starch and yeast cells from the gluten by hand for a period of at least 10 minutes, taking care to incorporate all shreds and particles of the original 20-gram sample.

Place the gluten ball in a graduated cylinder and add distilled water to a volume of 500 ml.   Again wash the gluten ball in this volume of water for another period of 10 minutes, in order to remove the yeast cells enmeshed therein, and add this solution to the first wash water. Thoroughly agitate this yeast and starch suspension which now totals 1,960 ml., and transfer 49 ml. to a 200-ml. Erlenmeyer flask and stopper to prevent evaporation.   Add 1 ml. of standard Carbol Fuchsin solution, made up according to the directions given in the U. S. Pharmacopoeia, to the 49 ml. of the suspension in the Erlenmeyer flask.   This gives a final dilution of 20 g. of dough in 2,000 ml. of solution, or a 1-to-100 dilution.

Allow the stained solution to stand four or five hours, or if convenient over night, before the counts are made.   This permits the color to stain the yeast cells thoroughly.   When examined under the microscope at the end of that period, it will be found that the yeast cells have taken on a deep, dark red color, while the starch cells are only colored a faint pink.   Any small particles of gluten material will also be colored a dark red but they can easily be differentiated from the yeast cells because of the regular contour and the cellular structure of the latter.   Likewise, it is possible to distinguish yeast cells from small or similarly shaped starch cells by the fact that the latter are always clear and translucent and show no cellular structure.

During the time that the 50-ml. portion of the "starch-yeast" suspension is being stained, place the gluten ball remaining from the

two washings in the graduate and add distilled water to bring the volume to 188 ml., which is about one-tenth the volume of the main suspension.   It is better to work with more concentrated solutions in this case because of the very small number of yeast cells still remaining in the gluten ball.   Add 0.2 ml. of concentrated hydrochloric acid to the solution and allow to stand over night.   At the end of the overnight period the gluten will be dissolved almost completely and upon shaking a colloidal suspension of the gluten is obtained, while the yeast cells are unchanged.   To 47 ml. of this suspension add 3 ml. of Carbol Fuchsin solution and let stand over night.   It is necessary to use considerably more of the staining solution in this case because the colloidal gluten seems to prevent the yeast cells from readily taking the stain. Even with this amount, the cells are colored only a faint pink, instead of a dark red, as in the case of the "starch-yeast" suspension.   However, the minute gluten particles remain colorless in this solution and show no cellular structure.

For this work in our laboratory a Thoma-Zeiss haemacytometer is used, and the counting chamber is a Thoma-Levy modification of the Burker apparatus with two Neubauer rulings.   Immediately before the actual counting, the flasks containing the stained solutions are shaken vigorously to bring into suspension all particles and cells which may have settled out.   With a stirring rod, drops are removed and one placed on each of the Neubauer rulings and one on the intervening moat.   The plano-parallel cover glass is placed in position with the thumb and forefinger and then one end of it is rapidly lifted up and down several times in order to expel any air bubbles and also to insure a uniform distribution of the solution.   It is essential that this precaution be taken.   The prepared slide is now allowed to set for a few minutes before the counting is started in order that the yeast cells may settle out upon the ruled surface of the counting chamber.   The counts are made with the high-power 4 mm. objective.

Each Neubauer ruling of the Thoma-Levy counting chamber is divided into nine large squares, each 1 mm. square.   The number of yeast cells in such a square is counted and an average taken of from 8 to 12 such counts.   Since there is a space of only 0.1 mm. between the cover glass and the ruled disc, it is necessary to multiply this average count by 10 to find the number of yeast cells in 1 cu. mm. of the solution.   This figure must be multiplied by 1,000 to convert to milliliters, and again by 100 because of the dilution of the solution.   Consequently, when the average count is multiplied by 1,000,000 the number of yeast cells present in one gram of the original dough is obtained.

Each of the larger square-millimeter squares in the Neubauer ruling is further subdivided into 16 small squares.   One of these small squares

completely covers the field of the microscope when the 4-mm. objective is used. In actual practice, therefore, the number of yeast cells in each such small square is counted and the number put down in its corresponding place in a table representing the larger sq.-mm. square. The sum of 16 such counts gives the number of cells in a sq. mm. It has been found preferable to count the cells in one or not more than two sq.-mm. squares, after which the counting chamber is cleaned and a fresh slide is prepared. This prevents errors due to evaporation of the solution, and it is for this same purpose that a drop of the solution was placed in the moat. In counting, the cells lying on the lines above and to the right are counted, never those on lines below or to the left. Yeast buds mature speedily in the dough into normal cells and exert the same influence on the dough as do the parent cells. For this reason, in counting, each bud that is large enough to be unmistakably recognized as such is counted as a single cell.

An Example of Counting

Each square represents a square millimeter on the Neubauer ruling. Thus, the 16 sets of figures inside the square represent the number of yeast cells in the 16 small divisions into which each square millimeter of the ruling is subdivided. Illustrated yeast cell counts follow:

4	2	3	3
6	7	12	11
7	4	7	8
6	7	5	7

Total 99 per sq. mm.

7	2	6	1
5	9	8	3
8	6	10	4
3	7	5	5

Total 89 per sq. mm.

4	4	8	4
6	2	7	4
6	8	3	10
4	3	7	4

Total 84 per sq. mm.

TABLE I

NUMBER OF YEAST CELLS IN SQUARE MILLIMETERS OF NEUBAUER RULING "STARCH-YEAST" SUSPENSION [1]

Individual Counter No. 1	Individual Counter No. 2
No. of cells	*No. of cells*
99	106
89	89
84	83
87	81
124	96
90	87
110	74
85	113
104	113
103	127
91	107
123	99
Average 99.1	98.0
Average of both counters: 98.5 per sq. mm.	

[1] It might be pointed out that two individual counters obtained remarkably close checks on their average total counts, as for example: 92.7 and 92.0, 33.3 and 35.8, 204.0 and 203.6, 52.3 and 52.5, 98.1 and 98.0.

Calculation of Number of Yeast Cells per Gram of Dough

The total number of yeast cells per gram of dough is obtained by adding the number of yeast cells found in the "starch-yeast" suspension and the number found in the "gluten-ball" solution. Since the "gluten-ball" solution is made up ten times more concentrated than the "starch-yeast" suspension, the count on the "gluten-ball" solution is divided by 10 before being added to the count obtained from the "starch-yeast" suspension. For example, if 98.5 cells per sq. mm. were found in the "starch-yeast" suspension, and 18.8 cells per sq. mm. in the "gluten-ball" solution, the total count would be 98.5 plus 1.88, which is 100.4 cells per sq. mm. Multiplied by 1,000,000 the result is 100,400,000 yeast cells per gram of dough.

Accuracy of Method

In order to check the accuracy of this method of counting yeast cells in doughs, a number of doughs were set in which the quantity of yeast was unknown to the persons making the counts. Since yeast in the form of compressed yeast varies in the number of cells per unit weight, it was necessary to determine by counting the number of cells per gram of the compressed yeast in order to make the necessary calculations.

TABLE II

The Accuracy in the Determination of the Quantity of Yeast in Doughs by Counting the Yeast Cells [1]

Type of dough	Cells per gram of compressed yeast used	Cells per gram of dough as determined by counting (average of 384 fields)	Percent yeast on basis of entire dough batch	
			Added	Found
	millions	*millions*	%	%
Laboratory	11,129	100.4	0.89	0.90
Laboratory	11,129	111.7	0.98	1.00
Commercial	11,129	95.8	0.86	0.86

[1] In making yeast cell counts on yeast cakes, it was of advantage to use iodine stain instead of Carbol Fuchsin. A suspension of yeast cells in water without other substances being present tended to form clusters in the presence of Carbol Fuchsin but not in the presence of iodine.

It was found that the size and character of the yeast cells varied considerably in different batches of compressed yeast. In some cases the cells were small and immature, in others larger and older; in some cases a large percentage of buds were present, and in others none. As a result, the number of cells per unit weight of yeast varied considerably, and it was necessary in order to calculate the percentage of yeast in an "unknown" dough to know the number of cells per unit weight in the original yeast.

Summary and Conclusions

A method for counting yeast cells in doughs with a high degree of accuracy has been presented.

This technique makes possible the study of the reproduction of yeast in the dough batch and factors that affect the growth. The use

of this method clarifies the uncertainties which have been controversial in nature.

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FACTORS AFFECTING THE GROWTH OF YEAST IN FERMENTING DOUGHS

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The development of a standardized and accurate method of counting yeast cells in doughs, Hoffman *et al.* (1941), has made possible the study of a number of problems concerning the growth of yeast in fermenting doughs. These problems are of both theoretical and practical interest to the baking technologist.

The Effect of Fermentation Time

A test dough containing the following ingredients was set at 80°F.: flour 960 g., water 555 g., sugar 35 g., salt 15 g., condensed milk 25 g., lard 20 g., and yeast 16 g. The yeast content was 1.67% based on the flour, or 0.98% on the entire dough batch. The dough was allowed to ferment at 80°F., and was punched regularly. Counts were made when the dough was mixed and after 2, 4, and 6 hours of fermentation.

TABLE I

EFFECT OF FERMENTATION TIME

	Yeast cells per gram of dough (average of 256 fields)
	millions
When mixed	98.05
After 2 hrs. of fermentation	98.45
After 4 hrs. of fermentation	124.85
After 6 hrs. of fermentation	133.60

The greatest amount of yeast-cell reproduction occurred between the second and fourth hours. This result is in accordance with the rate of gas production as shown in Figure 1.
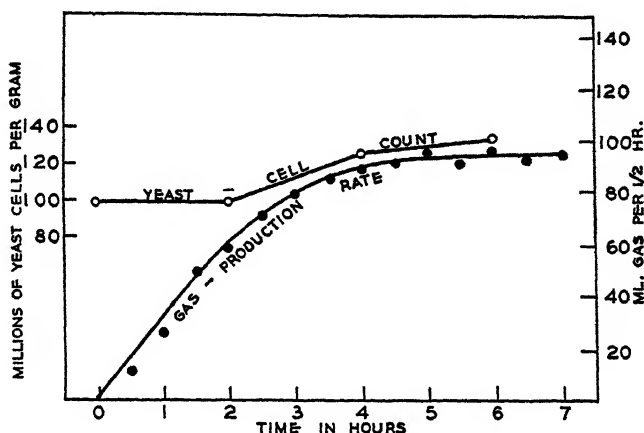
Fig. 1.   The relationship of gas-production rate and yeast-cell growth.

The Effect of the Quantity of Yeast

In this determination all variables such as time, temperature, and ingredients other than yeast were held constant as far as possible. The test doughs were used, the temperature maintained at 80°F., and the total fermentation time was six hours.

TABLE II

THE EFFECT OF THE QUANTITY OF YEAST ON YEAST GROWTH

| Percent yeast based on the flour | Yeast cells per gram of dough | | | |
	When mixed	After six hours	Increase	Percent increase
%	millions	millions	millions	%
0.5	29.8	56.2	26.4	88
0.75	39.7	64.1	24.4	61
1.00	52.1	82.6	30.5	58
1.25	67.3	101.1	33.8	50
1.50	82.0	122.5	40.5	49
1.75	93.3	135.6	42.3	45
2.00	115.7	149.9	34.2	29

It is interesting to note that the volume of baked loaves followed very closely the actual increase in yeast cells, as shown in Table II. The graphical representation of the relationship between the actual increase in yeast cells and the loaf volume of the resulting bread is given in Figure 2.

TABLE III

EFFECT OF QUANTITY OF YEAST ON LOAF VOLUME

Percent yeast based on flour	Fermentation time			Average loaf volume
	3 hrs.	3½ hrs.	4 hrs.	
%	cc.	cc.	cc.	cc.
0.5	1240	1440	1560	1414
0.75	1280	1470	1560	1437
1.00	1320	1540	1620	1493
1.25	1600	1620	1570	1597
1.50	1640	1640	1680	1653
1.75	1579	1710	1790	1690
2.00	1540	1650	1610	1600
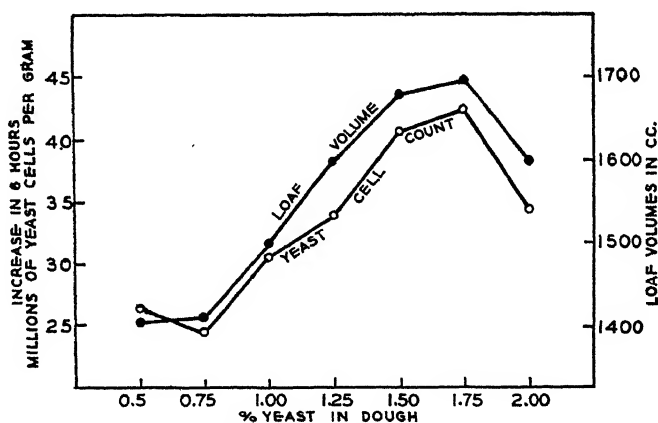
Fig. 2.   The relationship between the actual increase in yeast-cell count and the loaf volumes of the resulting loaves of bread.

Lindet (1910) showed that the smaller the quantity of yeast in a dough, the greater the percentage multiplication of cells in the dough. Geere and Geere (1922) also reported that when the quantity of yeast was decreased there was a considerably greater increase in the rate of growth of the yeast.   The results given above are in agreement, therefore, with previously published data.

The Effect of Ammonium Chloride and Ammonium Carbonate

The method of yeast cell counting is of special value in studying the effect of mineral salts on yeast growth.   Three test doughs were set: (1) control, (2) 0.048% ammonium chloride, and (3) 0.048% ammonium carbonate, with percentages based on the weight of the flour.   (The actual quantity of ammonia is very nearly the same in each salt.)   The doughs were fermented for six hours at 80°F., the

yeast cells counted, and the doughs panned, proofed, and baked. The resulting loaves were of course overaged, but the differences were still apparent.

TABLE IV

The Effect of Ammonium Chloride and Ammonium Carbonate on the Growth of Yeast as Related to Gas Production and Loaf Volumes

| Yeast nutrient | Yeast cells per gram of dough | | Total gas produced in six hours | Loaf volumes of baked bread |
	When mixed	After six hours		
	millions	*millions*	*ml.*	*cc.*
None	41.25	80.7	997.5	1540
0.048% NH₄Cl	40.0	101.5	1223.5	1670
0.048% (NH₄)₂CO₃	39.25	86.5	1187.5	1610

The dough containing ammonium chloride produced the greatest increase in yeast cells, produced the most gas, and the baked loaves were the largest in size. It would seem from this experiment that the ammonium compound as well as the ammonium ion plays a part in stimulating yeast growth.

The Effect of Ammonium Chloride on Yeast Growth when Different Quantities of Yeast Were Used

It has been shown that the greatest percentage of increase in yeast cell count took place when smaller quantities of yeast were used in the dough. This fact may perhaps be explained by the limiting quantity of nutritive material in the dough; and the more yeast present, the less available food material for each yeast cell. Therefore, yeast nutrients may show the greatest effect when the quantity of yeast used is less than normal.

In order to illustrate this point, four test doughs were set. Two contained the regular quantity of yeast with and without ammonium chloride, and two contained 25% less yeast with and without ammonium chloride.

TABLE V

The Effect of Ammonium Chloride on Yeast Growth when Different Quantities of Yeast Were Used

| Percent yeast based on flour | Ammonium chloride | Yeast cells per gram of dough | | | Percent increase |
		When mixed	After six hours	Increase	
%	*%*	*millions*	*millions*	*millions*	*%*
1.67	—	98.8	150.7	51.9	52.5
1.25	—	72.1	128.0	55.9	77.5
1.67	0.048	96.9	155.0	58.1	60.0
1.25	0.048	73.5	145.9	72.3	98.5

These results are shown graphically in Figure 3. They illustrate the fact that ammonium chloride exerts its greatest action when smaller quantities of yeast are used.
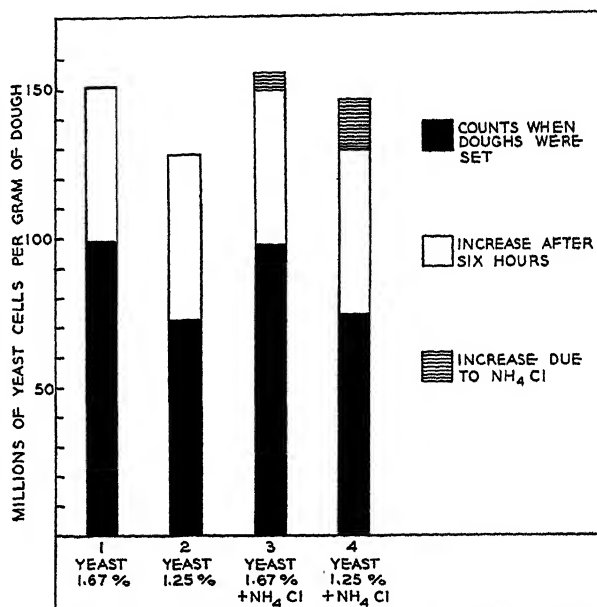

Fig. 3. The effect of ammonium chloride on yeast growth when the quantity of yeast was varied.

The Effect of Calcium Sulphate on Yeast Growth when Smaller Quantities of Yeast Were Used

As in the case of ammonium chloride, the effect of calcium sulphate was more pronounced when the quantity of yeast was smaller than the regular quantity used in the dough.

TABLE VI

THE EFFECT OF CALCIUM SULPHATE ON YEAST GROWTH

Dough	Percent yeast based on flour	Calcium sulphate	Yeast cells per gram of dough			Percent increase
			When mixed	After six hours	Increase	
	%	%	*millions*	*millions*	*millions*	%
No. 1	1.67	—	91.6	131.2	39.6	43.2
No. 2	1.50	—	79.2	110.5	31.3	39.5
No. 3	1.50	0.127	78.1	123.1	45.0	57.6

The data given in Table VI were selected for illustrative purposes from a large amount of experimental results. It had been determined

by baking tests that a decrease of 10% in the yeast, from 1.67% to 1.50%, plus 0.127% calcium sulphate (percentage based on the flour) gave finished loaves comparable in quality to the control loaves containing 1.67% yeast.

The Cumulative Effect of Two Yeast Nutrients

Ammonium chloride and calcium sulphate when used separately in doughs increased the rate of yeast-cell reproduction. When these two salts were used together there was a cumulative effect; that is, the effect of the two salts when used together was greater than the sum of the effects of the salts when used separately.

TABLE VII

THE CUMULATIVE EFFECT ON YEAST-CELL GROWTH WHEN AMMONIUM CHLORIDE AND CALCIUM SULPHATE WERE USED TOGETHER

Dough	Percent yeast based on flour	Ammonium chloride	Calcium sulphate	Yeast cells per gram of dough			Percent increase
				When mixed	After six hours	Increase	
	%	%	%	*millions*	*millions*	*millions*	%
No. 1	1.67	—	—	91.6	131.2	39.6	43.2
No. 2	1.50	—	—	79.2	110.5	31.3	39.5
No. 3	1.25	—	—	62.8	98.1	35.3	56.2
No. 4	0.92	—	—	50.6	73.0	22.4	44.3
No. 5	1.50	—	0.127	78.1	123.1	45.0	57.6
No. 6	1.25	0.048	—	59.5	116.4	56.9	95.6
No. 7	0.92	0.048	0.127	47.9	121.7	73.8	154.1

The increase due to calcium sulphate is obtained by subtracting the increase in yeast cells in dough No. 2 from the increase in yeast cells in dough No. 5 (45.0 − 31.3), giving 13.7 million yeast cells per gram of dough.

The increase due to ammonium chloride is obtained by subtracting the increase in yeast cells in dough No. 3 from the increase in dough No. 6 (56.9 − 35.3), giving 21.6 million yeast cells per gram of dough.

The additive increase due to both calcium sulphate and ammonium chloride is obtained by adding the increase in yeast cells due to calcium sulphate obtained above, 13.7, and the increase due to ammonium chloride, 21.6, giving 35.3 million cells.

The increase due to the combined action of ammonium chloride and calcium sulphate is obtained by subtracting the increase in yeast cells in dough No. 4 from the increase in dough No. 7 (73.8 − 22.4), giving an increase of 51.4 million cells. It was shown above that the additive increase was only 35.3 million cells. Therefore the cumulative effect (51.4 − 35.3) is 16.1 million yeast cells due to the combined action

These results are shown graphically in Figure 3. They illustrate the fact that ammonium chloride exerts its greatest action when smaller quantities of yeast are used.

Fig. 3. The effect of ammonium chloride on yeast growth when the quantity of yeast was varied.

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in a single dough of two yeast nutrients. These results are shown graphically in Figure 4.

The quantities of yeast used in conjunction with the yeast nutrients was determined by series of baking tests. It was found, for example, that 1.50% yeast and 0.127% calcium sulphate (dough No. 5) gave baking results comparable to the control containing 1.67% yeast. Dough No. 6, containing 1.25% yeast and 0.048% ammonium chloride,
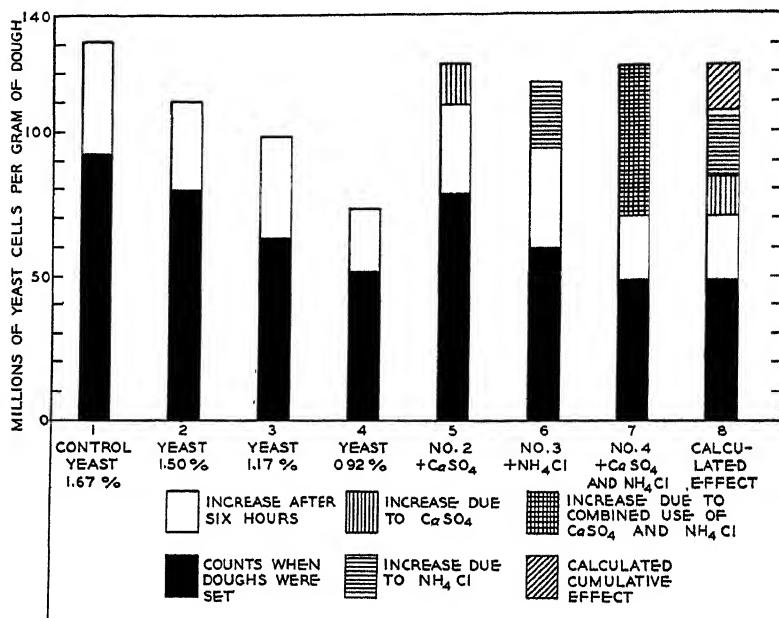
Fig. 4. The cumulative effect of ammonium chloride and calcium sulphate on yeast-cell growth.

produced bread of equal quality to the control. This was also true of dough No. 7, containing only 0.92% yeast and both calcium sulphate and ammonium chloride. The criterion in these experiments was the production of bread equal in quality to the control bread even though differences in amounts of yeast were substantial.

Summary

The following facts have been demonstrated by the method of yeast-cell counting:

1. Yeast definitely grows in the dough batch, the greatest amount of growth occurring between the second and fourth hours in a straight-dough formula.

2. 1.75% yeast based on the flour was found to give the optimum baking results under the conditions of this test.

3. Ammonium chloride was shown to be a better yeast nutrient than the closely related ammonium carbonate.

4. Ammonium chloride exerts its greatest action as a yeast nutrient when smaller quantities of yeast are used.

5. Calcium sulphate shows definite yeast-stimulating properties.

6. Ammonium chloride and calcium sulphate when used together in a dough show a cumulative nutritive effect on yeast.

<div align="center">Literature Cited</div>

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    1922   Composition of matter for increasing growth of yeast when mixed with dough. U. S. Patent No. 1,431,156.
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    1910   Sur le rôle de la levure en boulangerie. Compt. rend. 150: 802–809.

THE NUTRITIVE VALUE OF THE PROTEINS OF RICE AND ITS BY-PRODUCTS.[1] III. AMINO ACID CONTENT

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In studies of the biological value of the proteins of rice and its by-products, and of the effect of amino acid additions on growth (Kik, 1939, 1940), it was found that at a 5.5% protein level, the proteins of whole rice and those of polished rice had a lower biological value and a higher digestibility than those of rice bran and of rice polishings. Paired feeding experiments with rats showed that cystine, methionine, and lysine supplemented to a slight extent the proteins of whole rice and of polished rice fed at a 5%–6% level. Tryptophane, however, did not have any beneficial effect and cystine did not improve growth of the proteins of rice bran and rice polishings when fed at an 8% level.

Amino acids can be determined quantitatively in foods (Csonka, 1937a, 1937b), and the present investigation was therefore undertaken to determine the amino acid content of the proteins of whole rice, polished rice, rice polishings, and rice bran. Cystine, tryptophane, lysine, arginine, and histidine content were determined. All these amino acids are nutritionally essential, except cystine, which is only

essential when methionine is present in too low a level, and arginine, which can be partly synthesized in the body (Rose, 1938).

Considerable variation has been reported (Csonka, 1937a, 1937b), in the amino acid content of different varieties of wheat. In this paper data are presented on the amino acid content of different varieties of rice. Results are also submitted on amino acid determinations of the proteins of rice grown on plots treated with and without different fertilizers; such a study was included in order to find out to what extent, if any, the composition of the rice proteins could be effected by soil treatment.

Experimental Work and Discussion

Whole rice, white polished, rice, rice bran, and rice polish were purchased from a local mill and were of the Supreme Blue Rose variety. Other rice varieties and the rice grown on plots treated with and without fertilizer were obtained from the Rice Branch Experiment Station.[2] These rices were shelled with the aid of a shelling board, cleaned with a fanning mill, and milled to a fine flour.

Preparation of samples.—The samples were prepared as described by Csonka. A short outline of the slightly modified procedure follows: 25 g. of ground material was defatted by three half-hour extractions with 100 cc. of ether. This material was extracted three times for one hour with 1% NaCl solution at refrigeration temperature (6°–8°C.). This removed the water and salt-soluble nitrogen. This was followed by three 60% aqueous alcohol extractions in 100-cc. portions at room temperature, two of two hours' duration and one overnight. Then followed four 0.1% alkali extractions of one hour in 100-cc. portions and at room temperature. These alkali extracts were immediately acidified with dilute acetic acid.

The final extraction was with 100 cc. of a cold 20% HCl solution, the material was placed in the refrigerator for one hour, and stirred occasionally for dispersion of starch. At the end of the hour 150 cc. of 95% alcohol was added slowly with constant stirring for the starch precipitation and this material was centrifuged. This acid alcohol extraction was repeated. To the remaining material 300 cc. of 20% HCl was added in order to dissolve the starch, and later another 100 cc. The residue was hydrolyzed in 100 cc. of 20% HCl in an oil bath for 24 hours. The salt, alcoholic, alkali, and acid-alcohol extracts were combined and evaporated to a small volume on a water bath in evaporating dishes, then transferred to a 300-cc. Erlenmeyer flask. The material was finally hydrolyzed for 24 hours in an oil bath. The amino acids were determined in this hydrolysate, to which was added the hydro-

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lysate of the residue. The amino acids were determined by methods outlined by Block (1938).

Cyst'ne was determined according to the Sullivan method, and direct methods were employed for the determination of histidine, arginine, ana lysine. Histidine was quantitatively precipitated from the amino acid hydrolysate as its silver salt at pH 7.4 Arginine silver salt was precipitated between the pH range of 8.5 to 14.0, and lysine was precipitated by phosphotungstic acid from a solution containing 5% by weight of H_2SO_4. Tryptophane was determined according to the method of May and Rose as modified by Csonka (1937a and 1937b).

The distribution of nitrogen in the extracts included from 90% to 93% of the total nitrogen as can be seen from Table I. The salt-

TABLE I

NITROGEN EXTRACTED BY VARIOUS SOLVENTS, INDICATED AND EXPRESSED IN PERCENTAGE OF TOTAL RICE NITROGEN

Solvent	Whole rice	White rice	Rice from sulfur-treated plot	Rice from check plot
	%	%	%	%
1% NaCl	13.82	11.51	16.20	14.53
60% alcohol	3.66	5.75	4.00	3.00
0.1% alkali	44.22	40.85	40.00	40.00
Acid alcohol	3.98	5.33	4.70	3.70
Residue hydrolysate	25.90	27.60	28.50	32.00
Total yield	91.58	90.50	93.40	93.23

soluble type of protein constituted about 14%, the alcohol-soluble protein 4%, and the alkali-soluble up to 44%. The analytical results are given in Tables II, III, and IV.

Table II shows that whole rice and polished rice are not lacking in cystine, but they do have a low cystine percentage as compared to

TABLE II

TOTAL NITROGEN, AMINO ACID CONTENT AND MG. OF INDICATED AMINO ACID PER GRAM OF TOTAL NITROGEN IN WHOLE RICE, POLISHED RICE, RICE BRAN, AND RICE POLISHINGS, WITH CASEIN, CORN, AND WHEAT FOR COMPARISON

Material	Total nitrogen	Cystine		Tryptophane		Lysine		Arginine		Histidine	
	%	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.
Whole rice	1.23	0.090	73	0.074	60	0.260	212	0.254	223	0.064	52
Polished rice	1.02	0.073	72	0.066	65	0.280	275	0.251	247	0.059	58
Rice bran	2.14	0.137	64	0.096	45	0.443	207	0.344	161	0.090	42
Rice polishings	1.98	0.141	71	0.107	54	0.444	224	0.273	138	0.071	36
Casein [1]	15.00	0.300	20	1.950	130	7.120	475	3.540	236	2.340	156
White corn [1]	1.71	0.096	56	0.047	28	0.107	63	0.212	124	0.089	52
Wheat [1]	2.34	0.157	67	0.080	34	0.872	373	0.356	152	0.080	34

[1] These data are obtained from Csonka (1937 a and b, 1939).

casein and wheat. Rations containing whole rice or polished rice as the only source of proteins do not furnish enough cystine to support good growth and cystine addition has some supplementary effect (Kik, 1940). Tryptophane, arginine, and histidine content compare favorably with the content of those essential amino acids in wheat and corn. The lysine content of whole rice and polished rice, however, is much lower than that of wheat for which a supplementary effect of this amino acid has been reported (Mitchell, 1932).

A decrease of protein occurs in polishings of whole rice as seen by the lower percentage of nitrogen, which is accompanied by a small decrease in cystine, tryptophane, arginine, and histidine content.

From Table III it can be seen that there is considerable variation in the amino acid content of different rice varieties. This corresponds to the observation of Csonka (1937) on the amino acid content of wheat varieties.

<div align="center">TABLE III</div>

<div align="center">INDICATED AMINO ACID PER GRAM OF TOTAL NITROGEN IN VARIETIES OF RICE</div>

Variety of rice	Cystine	Trypto-phane	Lysine	Arginine	Histidine
	mg.	mg.	mg.	mg.	mg.
Arkansas 155	95	48	333	214	46
Shoemed	85	70	265	233	66
Arkada	68	69	256	263	37
Zenith	67	55	241	243	54
Fortuna	96	70	317	222	54

In Table IV are shown the results of amino acid determinations of rice grown on plots treated with and without different fertilizers. The rice in each group was of the same variety.

Although small increases were found in total nitrogen in all groups, considerable increases in individual amino acid contents were obtained in some groups.

Group I.—This group consisted of plots 7, 9, 14, and 12 which were treated with superphosphate (at the rate of 250 tons per acre), ammonium sulfate (at the rate of 100 tons per acre), Ammophosko (at the rate of 500 tons per acre), and the check plot (No. 12) which received no fertilizer. The total nitrogen of the rice grown on these plots was 1.38%, 1.30%, 1.31%, and 1.35% respectively. Superphosphate and Ammophosko increased the cystine content from 0.91% to 0.107% and 0.097% respectively, and the tryptophane content increased from 0.067% to 0.086% in the Ammophosko-treated plot and to 0.078% in the plot treated with superphosphate. The lysine percent increased from 0.303% to 0.322% in the Ammophosko-treated plot, to 0.366% in the ammonium sulfate-treated plot and to 0.323% in the superphos-

phate-treated plot. The arginine content increased from 0.256% to 0.352% in the Ammophosko-treated plot and to 0.354% in the ammonium sulfate-treated plot. Histidine was increased from 0.067% to 0.072% in the superphosphate-treated plot.

Group II.—Three plots were compared: Plot 2 received $NaNO_3$ at the rate of 150 pounds per acre and sulfur at the rate of 2,000 pounds per acre. Plot 3 received $NaNO_3$ at the rate of 150 pounds per acre and sulfur at the rate of 4,000 pounds per acre. Plot 4 was the check plot. The cystine content increased in Plot 2 ($NaNO_3$ and sulfur treated) from 0.076% to 0.088% and in Plot 3 ($NaNO_3$ and increased

TABLE IV

AMINO ACID CONTENT AND TOTAL NITROGEN OF RICE GROWN ON PLOTS TREATED WITH AND WITHOUT FERTILIZERS

Plot number and treatment of plot		Cystine	Trypto-phane	Lysine	Arginine	Histidine	Total nitrogen
		%	%	%	%	%	%
GROUP I							
7	Superphosphate	0.107	0.078	0.323	0.256	0.072	1.38
9	Amm. sulfate	0.092	0.066	0.366	0.354	0.067	1.30
14	Ammophosko	0.097	0.086	0.322	0.352	0.052	1.31
12	No fertilizer	0.091	0.067	0.303	0.256	0.067	1.35
GROUP II							
3	NaNO₃, 4000 S	0.097	0.087	0.305	0.231	0.085	1.35
2	NaNO₃, 2000 S	0.088	0.089	0.336	0.295	0.102	1.36
4	No fertilizer	0.076	0.080	0.247	0.276	0.096	1.29
GROUP III							
11	1000 S	0.076	0.089	0.344	0.290	0.079	1.33
12	2000 S	0.067	0.089	0.374	0.196	0.076	1.35
18	NaNO₃, 3000 S	0.066	0.082	0.330	0.329	0.092	1.33
14	NaNO₃	0.076	0.080	0.259	0.259	0.077	1.33
19	No fertilizer	0.073	0.079	0.257	0.186	0.085	1.28
GROUP IV							
27	Manure	0.076	0.075	0.338	0.249	0.080	1.23
29	No fertilizer	0.074	0.070	0.348	0.252	0.077	1.13

sulfur) to 0.097%. The tryptophane content was increased from 0.080% to 0.089% in the second plot and to 0.087% in the third. The lysine content increased from 0.247% to 0.336% in the second plot and to .305% in the third, both treated with $NaNO_3$ and sulfur. The arginine content was changed in the second plot only from 0.276% to 0.295%.

· *Group III.*—This group consisted of five plots, treated as follows: Plot 11 received sulfur at the rate of 1,000 pounds per acre, Plot 12 sulfur at the rate of 2,000 pounds per acre, Plot 18 $NaNO_3$ at the rate of 150 pounds and sulfur at the rate of 3,000 pounds per acre, Plot 14 $NaNO_3$ at the rate of 150 pounds per acre, and Plot 19 was the check plot. The total nitrogen was 1.33%, 1.35%, 1.33%, 1.33%, and 1.28%,

respectively. The tryptophane content was increased from 0.079% to 0.089% in the sulfur-treated plot. The lysine content increased from 0.257% to 0.344% in Plot 11, to 0.374% in Plot 12, and to 0.330% in Plot 18. The arginine content increased from 0.186% to 0.259%, 0.329%, 0.196% and 0.290% in the $NaNO_3$ and sulfur-treated plots.

Group IV.—This last group consisted of two plots, Plot 27 treated with manure at the rate of 500 pounds per acre and the check Plot 29. The total nitrogen increased from 1.13% to 1.23%. The cystine content increased from 0.074% to 0.076% in the manure-treated plot, the tryptophane from 0.70% to 0.75% and the histidine from 0.077% to 0.080%, changes too small to be considered significant.

From these data it is concluded that within the genetic make-up of the variety the composition of rice proteins can be influenced to a certain extent by the treatment of the soil (application of fertilizers).

Summary

Data are presented on the cystine, tryptophane, lysine, arginine, and histidine content of whole rice, polished rice, rice bran, and rice polishings. The amino acid content compared favorably with that for corn, wheat, and casein, except for tryptophane, lysine, and histidine, which were higher in casein. Differences were found in the composition of the proteins of rice varieties. Increases were obtained in cystine, tryptophane, lysine, arginine, and histidine content of the proteins of rice from plots treated with fertilizers (superphosphate, ammonium sulfate, Ammophosko, $NaNO_3$, and sulfur) as compared to the amino acid content of the proteins of rice from untreated plots.

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AN ACID–ALKALI METHOD FOR STAINING TRIBOLIUM EGGS IN FLOUR

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Two methods for separating *Tribolium* eggs from the bulk of a flour sample and subsequently staining them were described in an earlier paper.[1] By one of these methods the sample is bolted through a 6XX silk cloth and the eggs are concentrated in the overs, which are then digested with 5% sulfuric acid on a water bath. The digested mixture is filtered in a Buchner funnel, and the residue on the filter paper is treated with iodine solution, staining the eggs a golden- or yellow-brown and the starch and filter paper blue. By the other method the sample is shaken with a chloroform-toluol mixture in a special separatory funnel, and the eggs rise to the surface of the liquid where they may be decanted through a Buchner funnel. The filter paper is stained with iodine as previously noted.

While investigating the use of sulfuric acid as a digestant for flour overs, experiments were also conducted to determine the effects of dilute nitric acid, hydrochloric acid, and sodium hydroxide on *Tribolium* eggs. Sulfuric acid and hydrochloric acid appeared unreactive, whereas nitric acid and/or sodium hydroxide affected the eggs.

The purpose of this report is to present another method for staining *Tribolium* eggs in flour. The method is based upon the color manifested by the eggs after treatment with 5% nitric acid followed by 10% sodium hydroxide.

Effects of Acids on Overs and Eggs

The eggs used in these experiments were obtained from cultures of twenty *Tribolium* beetles in 50 g. of hard-wheat patent flour. The flour had previously been bolted through a 6XX silk cloth to remove all material which would not pass through the mesh. The cultures were kept in a darkened incubator for 48 hours before they were examined for eggs. The eggs were separated from the flour by bolting through a 6XX cloth.

To note the effects of acid, a definite number of eggs was transferred to a 250-cc. beaker, 30 cc. of acid was added, and the beaker, covered with a watch-glass, was placed on a boiling water bath for five to ten minutes. The temperature of the liquid in the beaker attained approxi-

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[1] B. L. Blumberg and C. W. Ballard, Food Industries 10 (No. 1): 36–38, 1940.

mately 80°C. After digestion, the material was filtered in a Buchner funnel and the eggs were examined before and after staining with iodine solution.

Tests conducted with 5% nitric acid, 5% hydrochloric acid and 5% sulfuric acid indicated that none of the acids destroyed the eggs, although after the nitric acid digestion and iodine stain the majority of eggs assumed a red-brown color (rather than the pale yellow produced by sulfuric acid or hydrochloric acid digestion), and the flour residue on the paper was more yellow-brown than blue or blue-brown. This, of course, interfered with the differentiation of the egg.

Effects of Sodium Hydroxide on Overs and Eggs

Tests paralleling those described above were carried out to determine the effects of varying concentrations of sodium hydroxide. The alkali tended to destroy the eggs rather quickly even in small concentrations. It was not possible to recover all of the eggs in a test when using only 1% sodium hydroxide at 80°C. for five minutes. Moreover, the alkali caused the flour to gelatinize during the digestion process, thus rendering filtration very slow.

Effects of Acid Followed by Alkali

While experimenting with the use of acid followed by alkali for digesting the overs, it was noted that eggs treated with nitric acid and then sodium hydroxide turned a distinct orange-red. Since the eggs could be fairly easily seen and the method was simple, this reaction was investigated more throughly in order to establish another procedure for staining *Tribolium* eggs.

The Acid-Alkali Method for Staining Tribolium Eggs

After a large number of trials under various conditions, the following acid-alkali procedure was found suitable for staining *Tribolium* eggs in patent wheat flour.

A small quantity (0.1 — 0.6 g.) of sample was placed in a glazed white porcelain crucible cover of 5 cm. diameter and 6–7 mm. depth. The sample was obtained by taking a small quantity directly or preferably by concentrating the bulk of a large sample by bolting through a 6XX cloth.

The sample was wetted with 1.5 cc. of 95% ethanol, and 5 cc. of 5% nitric acid (5.2 cc. conc. nitric acid plus 94.8 cc. dist. water) was added. Using a glass rod which had been flattened and bent at an angle at one end, the mixture was stirred thoroughly and the container, covered with a glass plate, was placed on a boiling water bath.

The mixture was heated on the bath for 7 to 8 minutes, removed, and then 3 cc. of 10% sodium hydroxide (w/v) was added. Using the glass rod, the alkali was thoroughly mixed with contents of the cover, and the liquid was then observed for the presence of opaque, orange-red, ovate structures. Occasionally, a low-power hand lens aided in the detection of the eggs and their differentiation from artifacts.

After the nitric acid digestion the liquid was yellowish, and upon addition of the alkali it turned yellow-orange. Undigested starch clumps appeared yellow to orange-red, but these were usually well dispersed in the final stirring and offered little difficulty. Bran particles, being normally yellow to brown in color, sometimes interfered. In doubtful cases, it was remedial to empty the contents of the crucible cover into a 9 cm. petri dish which was placed on a sheet of white paper and examined under a strong light. In this way the artifacts became almost invisible and the only readily apparent structures conforming to the description noted above were the eggs.

The starch and bran were also differentiated from the eggs by staining procedures. Thus when the contents of the crucible cover were filtered in a Buchner and the excess alkali was removed by washing, the starch, upon the addition of 1% aqueous iodine solution, turned blue. Similarly, when the material in the Buchner was covered with Ehrlich's triacid stain for about 30 seconds and the excess stain was removed by washing with water, the wheat hairs and many of the bran particles stained a distinct green. These observations were made by spreading the filter paper on a glass plate and examining it with the compound microscope (100 ✕) using transmitted light. In a limited number of experiments, the green coloration was helpful in distinguishing between bran tissues and foreign matter.

In the initial stages of this investigation, it seemed that the nitric acid and sodium hydroxide alone were capable of staining the eggs. At this time, however, it is thought that the flour itself also influences the reaction. Every time that the acid-alkali procedure was used in the presence of as little as 0.1 gram of flour, all of the eggs stained. In the absence of flour, the staining reaction was variable and occasionally only two or three of the five eggs stained. Until more data are obtained on the factors involved in this staining phenomenon, it seems advisable to conduct the test in the presence of at least 0.1 gram of flour residue.

Summary

The effects of dilute acid and alkali on *Tribolium* eggs have been noted and an acid-alkali staining procedure has been detailed. This procedure should be considered as a preliminary test which in certain re-

spects is simpler and more expedient than either of the iodine staining tests formerly devised.

Of the three methods—acid digestion, flotation, acid-alkali treatment—for staining *Tribolium* eggs in flour, the first probably will be found the most satisfactory for routine use on finely milled flours. When the acid-alkali procedure is used, the Buchner filtration and the addition of iodine solution are eliminated, but the results may not always be so readily interpreted by the inexperienced observer.

STUDIES IN FLOUR GRANULARITY

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The effect of the degree of fineness of flour on flour quality and its suitability for various purposes has not been studied as much as the subject would seem to warrant. One reason for this is the difficulty in measuring accurately flour granularity and thus in differentiating between flours of slightly different degrees of fineness which might nevertheless have an effect in breadmaking and other processes.

The difficulty of making such measurements was discussed by Kent-Jones, Richardson, and Spalding (1939), who suggested a simple technique for making such measurements. The technique suggested had been used for determining soil particle size and is based upon the application of Stokes' law. It was not claimed that the idea was original, as Gründer (1932) and others had suggested a similar method, but the actual procedure suggested was found to be extremely useful and one which could be easily applied to routine work.

A uniform suspension of a small quantity of flour, such as one gram, in about a litre of petroleum, the physical constants of which, such as specific gravity, viscosity, etc., were known, is made and allowed to settle. The decrease in the cloudiness of this dilute petroleum suspension of flour is measured at a fixed depth by the increase in the intensity of a focused beam of light which shines through the suspension and thus falls on a photoelectric cell. This is recorded by the rise in a galvanometer reading.

For full details of the method, the paper quoted should be consulted. It is not suggested that the results are absolute and there is evidence to suggest that they are somewhat empirical in nature. For instance, in the apparatus used, the distance between the top of the petroleum and

the beam of light is 15 cm. Theoretically this distance might be anything convenient and it should not affect the final results. In practice, if a different depth is used, slightly different results are obtained. A number of factors have to be considered, such as whether Stokes' law can be applied in such circumstances, the effect of the petroleum on the flour, the influence of temperature, etc. The work which has been done with this method, however, suggests that errors introduced by these variations are not serious.

The main point is that in actual commercial experience the results have proved to be particularly illuminating and typical results on flours obtained by this method are given in this paper. In dealing with the sizes of flour particles, there is a fairly narrow range and what is wanted is some method for differentiating within this range. If, for example, the sizes of coarser particles, such as semolina, were wanted, it would be necessary to use a liquid other than petroleum in which to suspend and settle the solid material.

Experimental Results—British-Milled Flours

As Kent-Jones, Richardson, and Spalding (1939) have pointed out, the effect of climatic conditions unfortunately renders ordinary sieving tests on flour unreliable, as the same flour will dress through differently from day to day according to the temperature and relative humidity of

TABLE I

SEDIMENTATION VS. SIEVING TESTS

	211–152 μ	152–124 μ	124–89 μ	89–66 μ	66–25 μ
	%	%	%	%	%
SAMPLE I					
Sieving test	0.5	0.7	7.3	13.5	78.0
Sedimentation method (Kent-Jones, Richardson, and Spalding)	—	—	8.7	17.6	73.7
SAMPLE II					
Sieving test	0.2	0.3	2.5	9.5	87.5
Sedimentation method (Kent-Jones, Richardson, and Spalding)	—	—	4.2	13.0	82.8

the air. With certain materials which are more crystalline in nature, this difficulty does not arise and then sieving can be done with considerably more accuracy.

An opportunity arose to check out the present method of measuring particle size when an inorganic chemical which was not affected by cli-

matic condition was submitted both to this procedure and to careful sieving with standard sieves. The particle sizes happened to be of similar range to flour, and the results of the two methods are given in Table I. To make this comparison the range in size of the sieving test was worked out and the particle size from our experiment calculated within these groups. The agreement is reasonable and confirms the usefulness and accuracy of the method when used with such a difficult substance as flour.

In routine practice the determination of flour granularity with the apparatus described by Kent-Jones, Richardson, and Spalding is a simple matter.

Stokes' law is given by the equation:

$$V = \frac{(\zeta_1 - \zeta_2)g \times D^2}{18\eta},$$

where V = velocity of the falling sphere

　　ζ_1 = density of the falling particle (In this case the flour and the difference between different kinds of flour is not serious.)

　　ζ_2 = density of the liquid—in this case the petroleum

　　D = diameter of the falling particle

　　η = viscosity of the liquid, *i.e.*, of the petroleum

Now, if the distance between the surface of the liquid and the beam of the light is h, then

$$V = \frac{h}{t},$$

t being the time taken for a given particle to fall from the surface of the liquid to where the beam of light shines through the liquid.

Hence, combining the two equations we get

$$D = \sqrt{\frac{h \times 18\eta}{(\zeta_1 - \zeta_2)g} \times \frac{1}{t}}.$$

At a given temperature ζ_1, ζ_2 and η are constant so that the equation becomes

$$D = \sqrt{\frac{k}{t}},$$

where k is the constant for the apparatus when flour and petroleum is used at a fixed temperature. In actual practice k is determined once and for all from the constants of the flour and the liquid. Those particles which commenced their travel from the surface of the liquid will be the last of that particular size to pass the beam of the light, and hence it is possible from the equation to construct a table showing the

times at which all particles of a given size will have passed below the
light beam.

The apparatus is used at 15° C. and, with the distance from the
top of the liquid to the light beam being 15 cm., the value for k is
0.00195.

Hence the equation is

$$D = \sqrt{\frac{0.00195}{t}}.$$

Therefore at any stated time we can calculate D, the diameter of the
particles which have passed the beam.

Time in seconds	Diameter of particles in microns
15	114.0
25	88.2
45	65.8
60	57.0
120	40.3
240	28.6
600	18.0

The rise in the galvanometer reading between two times t_1 and t_2,
for example, is due to the removal of particles between the sizes indi-
cated by a graph constructed from the above table. In practice, all that
it is necessary to do is to multiply the rise in the galvanometer reading
by the mean of the diameters in question and the figures obtained will
be proportional to the mass of particles within that range. Assuming
limits for the largest and smallest particles in the material in question,
the relative masses can be expressed as percentages and thus a very
useful picture given of the granularity of the material.

An example will make this clear. In the writer's laboratory printed
forms exist and all the assistant has to do is to weigh out one gram
of flour, see that the temperature, the height of the liquid, etc., are cor-
rect, the galvanometer set, the stirring commenced, and then the watch
started as the stirring is switched off (Fig. 1).

The form is as shown and an actual result is illustrated. Readings
are taken at the times indicated and the differences noted. These dif-
ferences are multiplied by factors on the sheet (*i.e.*, mean of the particle
sizes involved). The products so obtained are added together and the
groups expressed as a percentage of the total. The form is so arranged
that calculations can be made for obtaining the result in the four groups
105—55 μ, 55—35 μ, 35—25 μ, and 25—15 μ, as well as the percentages
above and below 45 μ.

LABORATORY GRANULARITY FORM

Mill . . . . Date . . .
Flour Kansas 2nd Sizings . . . . Lab. No. 2657 SL

Time	Gal. Rdg.	Difference x Average Size	% Total	Difference x Average Size	% Total
15 SECONDS 17.5 SECS.	116 17.6				
20 SECONDS	19.6				
25 Seconds	19.5	1.3 x 8.0 = 10.4	31/105/55µ	1.9 x 7.5 = 14.3	38 Above 45µ
30 Seconds	19.3				
45 Seconds	18.9				
1 MINUTE	18.3				
1½ Minutes	17.9	1.4 x 4.5 = 6.3	19/55/35µ		
2 Minutes	17.3				
2½ Minutes	16.9				
3 Minutes	16.3	2.7 x 3.0 = 8.1	24/35/25µ		
4 Minutes	15.2				
5 MINUTES	14.2				
6 Minutes	12.6			7.9 x 3.0 = 23.7	62 Below 45µ
7 Minutes	11.8				
8 Minutes	11.5	4.4 x 2.0 = 8.8	26/25/15µ		
9 Minutes	11.2				
10 Minutes	11.0				
11 Minutes	10.8				
12 Minutes	10.5				
13 Minutes	10.1				
14 MINUTES	9.8				
TOTAL		33.6	100 %	38.0	100%

OPERATOR . . . .

Figure 1.

In the original paper of Kent-Jones, Richardson, and Spalding examples of the results obtained with this method on ordinary flours and finely dressed cake flours were given. Since that paper was compiled many hundreds of such particle-size analyses have been carried out, especially on flours which it was suspected might show interesting results from this standpoint. Table II gives some particle-size analyses on national straight-run flours as are at present being made in Great Britain. In Great Britain only straight-run flours may be made and these are milled to a 72% extraction. All the flours in question are ordinary commercial flours made from blended wheats. In this table the results

TABLE II

PARTICLE-SIZE ANALYSES OF VARIOUS STRAIGHT-RUN FLOURS

Mill No.	105–95 μ	95–85 μ	85–75 μ	75–65 μ	65–55 μ	55–45 μ	45–35 μ	35–25 μ	25–15 μ
	%	%	%	%	%	%	%	%	%
1	3	2	6	9	11	10	12	20	27
2	3	6	7	8	12	13	15	23	13
3	4	6	8	10	12	12	12	19	17
4	2	6	11	11	12	11	12	20	15
5	6	5	5	8	12	10	11	20	23
6	4	8	7	7	8	12	12	21	21
7	2	5	5	10	11	12	15	23	17
8	5	4	7	9	10	11	11	18	25
9	3	5	8	12	12	11	12	19	18
10	5	7	8	9	11	12	10	22	16
11	2	5	9	10	12	11	12	24	15
12	5	6	7	13	12	13	12	18	14
13	3	4	6	8	11	11	13	23	21
14	5	11	12	15	14	11	10	16	6
15	6	6	10	14	14	13	9	15	13
16	0	2	˙7	8	9	10	12	22	30

Mill No.	Greater than 45 μ	Less than 45 μ	105–55 μ	55–35 μ	35–25 μ	25–15 μ
	%	%	%	%	%	%
1	41	59	31	22	20	27
2	51	49	36	28	23	13
3	52	48	40	24	19	17
4	53	47	42	23	20	15
5	46	54	36	21	20	23
6	46	54	34	24	21	21
7	45	55	33	27	23	17
8	46	54	35	22	18	25
9	51	49	40	23	19	18
10	52	48	40	22	22	16
11	49	51	38	23	24	15
12	56	44	43	25	18	14
13	43	57	32	24	23	21
14	68	32	57	21	16	6
15	63	37	50	22	15	13
16	36	64	26	22	22	30

are first given in groups, the limit of which is 10 μ. It was found particularly useful in the paper mentioned also to give the percentage of particles larger and smaller than 45 μ. Since then a greater number of samples have been examined and it has been found that another and more useful method of presenting the results is to give the percentage masses of particles between the following four groups: 105—55 μ, 55—35 μ, 35—25 μ, and 25—15 μ.

In Table II 16 such flours are given and it will be seen that there are some interesting variations. A few notes on these flours may be of particular interest. Mill No. 1 shows a comparatively small number of

larger-size particles (particularly the 105—55 μ group) and a comparatively large number of the smaller-size particles, 25—15 μ. There is evidence in this mill of overgrinding which results in the flour yielding a thirsty but "dead" and inelastic dough. Mill No. 2 may be taken as a typical or average mill and it will be seen that the results are very similar to mills No. 3, 4, 7, 9, 10, 11, and 12. All these mills produce normal flours. It was suspected that Mill No. 5 had a tendency to overgrind and this is revealed by the nature of the flour produced. The particle size analysis tends to confirm this suspicion of overgrinding as there is a comparatively high percentage of particles in the 25—15 μ group.

On the other hand, mill No. 6 has a rather high percentage of small sized particles but the flour always gives a pleasingly elastic dough and shows no tendency to the "dead" type of dough usually associated with overground flour. Mill No. 6 happens to be a new mill with a large amount of roller surface. Mill No. 8 is very similar in dough characteristics to Mill No. 6—*i.e.*, is completely satisfactory—and the particle size distribution is not unsimilar. The same applies to mill No. 13, which is possibly the most up-to-date and newest mill erected in Great Britain. The result from this mill particularly indicates that it cannot be assumed that, because there is a compartively low number of large size particles (105—55 μ) and a tendency towards a high percentage in the smallest-sized group, there is indication of over-grinding.

Mill No. 14 is particularly interesting as the flour is so coarse, but this is a mill which grinds only a mixture of Manitoba and Yeoman wheat. The latter wheat breaks down in the mill in a similar granular way to Manitoba. The same applies to some extent to mill No. 15, both of them being country mills.

Mill No. 16 is interesting and would certainly seem to show overgrinding. This is believed to be the case, but it is not an English mill—actually it is situated in Africa.

These results are not conclusive, but a small percentage of particles in the largest size group and a large number in the smallest size group may, and often does, indicate overgrinding damage with consequent ill effects on the baking quality. It is possible, however, to have conditions approaching this particle size distribution with new mills of long surfaces, the flour of which does not have these defects. With normal average mills, however, such a particle size distribution would be looked upon with suspicion.

Table III gives some results with individual flours from reduction rolls and, as examples, the flours from the *A, B, C* and *D* reduction rolls are given from a number of different mills. It is necessary to

TABLE III

GRANULARITIES OF FLOURS FROM REDUCTION ROLLS

Mill No.	Above 45 μ	Below 45 μ	105–55 μ	55–35 μ	35–25 μ	25–15 μ
	%	%	%	%	%	%
			FLOUR *A*			
2	39	61	27	23	19	31
17	37	63	28	20	19	33
18	46	54	35	22	25	18
I	40	60	30	21	18	31
II	36	64	27	19	23	31
III	39	61	30	20	23	27
		ENGLISH WHEAT				
I	33	67	28	18	25	29
II	41	59	31	18	17	34
		MANITOBA WHEAT				
II	65	35	52	25	11	12
III	58	42	48	22	17	13
			FLOUR *B*			
2	53	47	42	22		17
17	42	58	31	22	18	29
18	52	48	42	21	23	14
I	47	53	36	21	11	32
II	46	54	38	18	21	23
III	56	44	45	22	18	15
		ENGLISH WHEAT				
I	33	67	28	18	25	29
II	41	59	31	18	17	34
		MANITOBA WHEAT				
II	65	35	52	25	11	12
III	58	42	48	22	17	13
			FLOUR *C*			
2	53	47	40	22	18	20
2 (Another sample)	59	41	48	21	13	18
17	57	43	47	20	13	20
	56	44	45	25	17	13
			FLOUR *D*			
2	39	61	26	31	25	18
18	65	35	51	25	12	12
I	65	35	51	25	13	11
II	46	54	32	26	19	23
III	82	18	68	23	6	3
		ENGLISH WHEAT				
I	48	52	31	32	15	22
II	30	70	22	19	23	36
		MANITOBA WHEAT				
II	58	42	40	33	13	14
III	53	47	35	34	19	12

remember how these particular flours are made. Flour *A* is obtained by the reduction of clean coarse semolina—possibly termed " first midds coarse " in America obtained from the purifiers fed from the early break rolls. *B* is obtained from the fine and clean semolina (first midds fine) from the same purifiers. The feed to *C* roll is mainly the dunst sheets from *A* and *B* flour centrifugals mixed sometimes with fine middlings, *i.e.*, very fine clean semolina. Flour *D* is more often than not obtained by reducing the dunst sheet of *C* together with some fine middlings.

With *A* flour, six mills are given, three of them (Roman letters) being taken from the previous paper. Where necessary, the particle sizes into the four group divisions are recalculated. These results are given so that later comparison can be made with certain American mill streams. With normal mixtures there is very little comment, except that mill No. 18 is distinctly coarser than the others. Illustrations are given of the particle sizes of *A* flour when certain mills were grinding either all English wheat (very soft), or all Manitoba.

The results of *B* flour show this flour to be distinctly coarser than *A*. Again mill No. 18 has a particularly small number of particles in the smallest size group. The same difference between English and Manitoba is clearly seen.

C flour calls for no special comment, but again mill No. 18 tends to have a particularly small percentage of the smallest size group.

With respect to *D* flour, marked variations are observed and this is typical of the flours lower down the mill. Much will depend upon the particular way of grinding the mill in question has, and it is, therefore, suggested that such determination of particle size might be extremely useful in checking up the routine work of mills and in comparing the running of different mills in, for example, one large group, provided, of course, they were working on similar wheats. There is a fairly marked difference in the case of the Mills I and II grinding English wheat. A study of these results confirms that the division into the two groups larger and smaller than 45 μ is not as helpful in differentiating as the four divisions which are now normally employed.

Results with American-Milled Flours

The writers hoped to receive in time for this communication the various flour streams of a Continental mill, but, owing to war disturbances, this has not been possible. Thanks, however, to the courtesy of American confreres a series of American flours of three types, Kansas, soft, and spring, has been submitted to this method of particle size analysis and the results are given in Table IV.

TABLE IV

GRANULARITY TESTS ON SAMPLES RECEIVED FROM GENERAL MILLS INC.

Sample	Lab. No.	105–55 μ	55–35 μ	35–25 μ	25–15 μ	Above 45 μ	Below 45 μ
			KANSAS				
1st midds fine	Z1.5740	29	26	23	22	36	64
1st midds coarse	5741	29	22	23	26	34	66
2nd midds coarse	5742	30	23	23	24	37	63
2nd stream from 2nd midds	5743	40	25	24	11	54	46
3rd midds	5744	32	23	23	22	40	60
4th midds	5745	58	24	12	6	70	30
5th midds	5746	50	25	15	10	63	37
6th midds	5747	37	26	22	15	52	48
7th midds	5748	39	32	19	10	56	44
Break midds	5749	40	25	24	11	56	44
1st break	5750	16	20	32	32	23	77
2nd break	5751	12	21	32	35	20	80
3rd break	5752	24	23	28	25	30	70
Fine first tailings	5753	41	21	18	20	48	52
1st grade low tailings	5754	29	32	20	19	43	57
1st sizings	5755	24	21	29	26	30	70
2nd sizings	5756	31	19	24	26	38	62
Stone stock	5757	65	17	12	6	72	28
Good suction	5758	19	17	23	41	30	70
Rescalp reel	5759	34	24	21	21	42	58
Kansas wheat flour	5760	43	26	25	6	58	42
			SPRING				
1st midds	5774	34	32	18	16	50	50
2nd midds	5775	31	28	22	19	42	58
2nd stream from 2nd midds	5776	46	23	18	13	57	43
3rd midds	5777	23	29	25	23	34	66
2nd stream from 3rd midds	5778	22	18	25	35	30	70
4th midds	5779	37	36	14	13	57	43
5th midds	5780	50	28	12	10	66	34
6th midds	5781	57	27	11	5	75	25
7th midds	5782	50	34	9	7	74	26
1st break	5783	51	33	11	5	69	31
2nd break	5784	32	27	28	13	49	51
3rd break	5785	29	31	24	16	41	59
1st sizing	5786	32	28	21	19	42	58
2nd sizing	5787	30	27	22	21	42	58
1st stone stock	5788	56	22	15	7	68	32
2nd stone stock	5789	59	25	11	5	77	23
1st one tailing	5790	42	33	16	9	62	38
Coarse one tailing	5791	36	29	19	16	47	53
One low grade	5792	52	25	14	9	72	28
1st scalp	5793	34	23	20	23	42	58
Good suction	5794	13	22	24	41	23	77
Finished spring wheat patent	5795	45	30	9	16	61	39

TABLE IV—*Continued*

Sample	Lab. No.	105–55 μ	55–35 μ	35–25 μ	25–15 μ	Above 45 μ	Below 45 μ
				SOFT			
1st midds	5761	19	16	24	41	22	78
2nd midds	5762	29	18	24	29	34	66
3rd midds	5763	29	22	21	28	35	65
4th midds	5764	37	23	21	19	48	52
5th midds	5765	33	24	18	25	46	54
1st sizing	5766	12	9	29	50	15	85
2nd sizing	5767	4	21	23	52	12	88
1st break lower	5768	9	17	27	47	16	84
1st break upper	5769	7	11	25	57	10	90
2nd break lower	5770	12	17	27	44	14	86
2nd break upper	5771	29	11	20	40	32	68
3rd break upper	5772	9	14	23	54	12	88
Soft wheat flour	5773	13	14	25	48	17	83

Owing to the differences between American and British milling systems it is difficult to make proper comparisons. The author is indebted to Mr. J. Lockwood of Messrs. Henry Simon, Ltd., for the following information. Mr. Lockwood is conversant with both British and American milling systems. He states that while it is comparatively easy to make a comparison between a British and a German diagram, the American is so different that too close a comparison might be misleading. In a broad sense, however, the following comparison is suggested:

1st midds fine	= B
1st midds coarse	= A
2nd midds coarse	= B mixed with A (from the third break purifier)
2nd stream from 2nd midds	= Coarse C
3rd midds	= Fine B (from coarse midds purifier)
4th midds	= G (the dunst from the BMR)
5th midds	= H
6th midds	= J
7th midds	= K
Break midds	= BMR
1st break	= 1st break
2nd break	= 2nd break
3rd break	= 3rd break
Fine first tailings	= B2
1st grade low tailings	= L
1st sizings	= Coarse A
2nd sizings	= B2
Stone stock	= Fine C
Good suction	= Exhaust flour

Naturally, the soft flours have, generally speaking, only a small percentage of particles in the large size group and a large number in the small size group, and, in this respect, it is interesting to compare the straight-run flours, namely the Kansas wheat flour (Lab. No. Z.1.5760) with the soft-wheat flour (Lab. No. Z.1.5773) and the spring-wheat flour

(Lab. No. Z.1.5795). Broadly speaking, the difference between the Kansas and the spring patent is not marked.

In general the size distribution of the top-grade flour in the case of the Kansas and spring varieties is not unlike the top reduction rolls in English mills grinding Manitoba wheat, except that there is a tendency toward coarseness in the English mills. The 6% of particles in the 25–15 μ group in the case of Kansas (total flour) is not in line with the results of the various mill streams making up the straight-run flour, but the results are actually those obtained with the samples sent. The same is true of the percentage of particles in the 35–25 μ group in the case of the spring patent (Lab. No. Z.1.5795).

Summary and Conclusions

Numerous mill streams, both British and American, have been submitted to analysis for particle-size distribution by the method suggested by Kent-Jones, Richardson, and Spalding, or at least by the routine way this method has been used in commercial practice. Examples have been given of how useful the application of this test can be in milling practice. The routine performance of the test is extremely simple and the information obtained is presented in a simple way which is of commercial value.

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THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON THE MALTING QUALITY OF FOUR BARLEY VARIETIES [1]

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The regulation and control of the environment during malting and the varietal response of barley to such conditions are important from the standpoint of obtaining desired types of malt and efficiency in plant operations. A thorough study of different malting conditions would require a very large number of samples, because so many combinations of environmental conditions may be obtained. Temperature during steeping and malting, moisture content of steeped and germinating barley, and the period of growth may be studied in numerous combinations. The results reported in this paper deal only with conditions that were held constant throughout the malting process. For example, when lots were malted at a prescribed moisture, they were held as constant as experimental technique would permit. If a barley was steeped to a high moisture content, it was maintained at this high level throughout the growing period. Likewise, when a sample was malted at 16°C. (60.8°F.), it was held at that temperature throughout the prescribed time. The writers do not overlook either the need or the desirability of having variability in these conditions during the malting process; but they do feel that a study of controlled and constant conditions is the first approach to the problem. Furthermore, the interpretation of results obtained would be made more difficult under variable or fluctuating conditions. Nevertheless, the study of fluctuating conditions, changed systematically or periodically, might offer a fuller expression of their influence on malt quality.

Plan of Experiment

The plan of this experiment was to study four barley varieties malted at two moistures (43% and 49%), four growth periods (2, 4, 6, and 8 days), four malting temperatures (12°, 16°, 20°, and 24°C.), and one steep temperature (16°C.). With a series of growth periods and malting temperatures, trends and influences can more readily be discovered and interpreted by the use of such graphs as Figure 1.
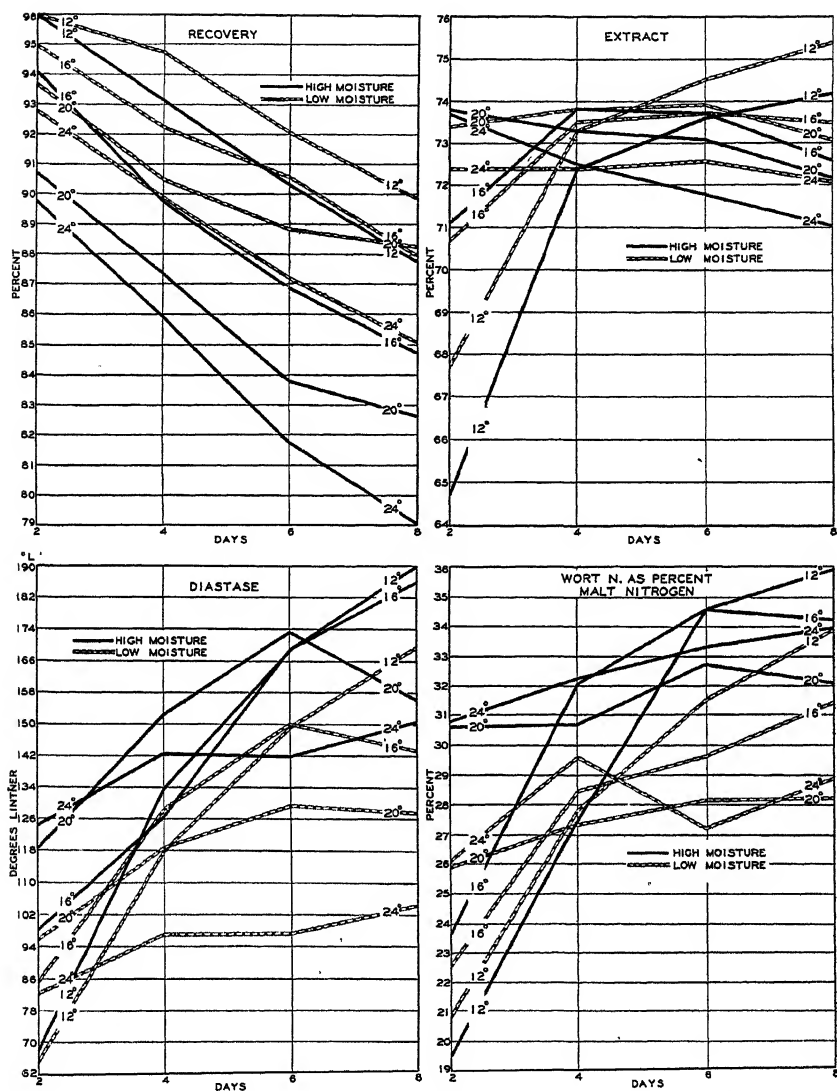
Fig. 1.   Influence of malting temperature, moisture, and growth time on the malting quality of four barley varieties.   Steep temperature, 16°C.; malting temperature, 12°, 16°, 20°, and 24°C.; moisture 43% and 49% for low (broken line) and high (solid line) respectively; and 2, 4, 6, and 8 days' growth time.   *Upper left:* recovery in percent   *Upper right:* extract in percent.   *Lower left:* diastase in degrees Lintner.   *Lower right:* wort nitrogen as percent of malt nitrogen.

The scope of the experiment was extended at one point by the use of two of the varieties at the additional steep temperature of 12°C. Thus, 32 different combinations of environmental conditions were used for four varieties, and an additional 32 conditions in the case of two varieties where another steep temperature was added. The plan of the experiment may be had in outline from Tables II to XII. Four varieties, of contrasting malting properties, were chosen in order to study their responses to different malting procedures. Environmental conditions, and varieties studied in this experiment represent only a small number of those possible.

Materials and Methods

The four varieties used were (1) Oderbrucker (Wisconsin Pedigree 5-1) (C.I. 4666),[2] (2) Wisconsin Barbless (Pedigree 38) (C.I. 5105), (3) Peatland (C.I. 5267), and (4) Chevron (C.I. 1111). The first two varieties have been used in a regional study by Dickson *et al.* (1935) and have been described briefly. Peatland and Chevron are rough-awned, low-yielding barleys imported from Switzerland. Aamodt and Johnston (1935) have described Peatland while Shands (1939) has pointed out the possibilities in using Chevron as a breeding stock. The lot of Oderbrucker barley was produced at the West Hill Farm at Madison, Wis., in 1936. The Wisconsin Barbless barley was grown near Manitowoc, Wis., in 1937. Peatland and Chevron were produced in yield plot trials by B. D. Leith in 1937 at the West Hill Farm. The kernel weights in milligrams, dry basis, for these varieties were 27.62, 25.90, 24.85, and 24.89, respectively. Oderbrucker usually has lower kernel weight than Wisconsin Barbless when grown under similar conditions. The barley nitrogen percentages in the same order were as follows: 2.08, 2.07, 2.28, and 2.49.

The barleys were steeped, germinated, and dried in screen-bottomed cans 5 inches in diameter and 4 inches high. Steeping was done in rectangular pans placed in the malting chamber where the desired temperature was maintained.

The malting chamber consisted of a modified Minneapolis seed germinator equipped with a small fan set to blow against a fine water spray in order to provide circulation of humidified air. Temperature in the chamber was controlled by circulating cold water in the surrounding water jacket, and a thermostated heating bulb within the chamber. The jacket temperature was maintained at approximately 1°C. below the desired temperature and accurate control was accomplished by the heater bulb.

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[2] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

The kiln or drier had a capacity of 18 cans on a single shelf. The samples were placed over holes with beveled edges, so that the exhaust fan on the top of the drier produced a movement of heated air through the malt samples. Heat was supplied by several independently operated resistance coils which gave considerable flexibility in temperatures obtainable. Laboratory air was drawn in through holes in the bottom of the drier and passed over the heating elements to acquire the desired temperature, and then up through the malt samples.

TABLE I

THE MOISTURE CONTENT OF STEEPED BARLEY, GREEN MALT, AND DRIED MALT OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

| Variety | Malting moisture | | | |
| | Low | | High | |
	16°C. steep	12°C. steep	16°C. steep	12°C. steep
	STEEPED BARLEY			
Oderbrucker	42.1	42.9	48.1	47.6
Wis. Barbless	43.7	44.3	48.9	48.5
Peatland	42.7	—	49.0	—
Chevron	42.3	—	49.2	—
	GREEN MALT			
Oderbrucker	43.5	43.6	49.6	49.8
Wis. Barbless	43.3	43.5	49.2	49.4
Peatland	43.0	—	49.3	—
Chevron	43.0	—	49.2	—
	DRIED MALT			
Oderbrucker	6.7	6.9	6.6	6.7
Wis. Barbless	6.8	7.0	6.8	7.0
Peatland	6.8	—	6.5	—
Chevron	7.0	—	6.5	—

Samples of the barleys weighing 200 g. were placed in the malting cans and steeped at 12° and 16°C. for a number of hours, as determined by a preliminary steep. Approximately 6,400 g. of both Peatland and Chevron were subdivided into 32 lots of 200 g. each by means of a Boerner divider and were stored until used for malting. Oderbrucker and Wisconsin Barbless lots were subdivided by the same means into 64 lots of 200 g. each. After division, each portion was weighed and made to exactly 200 g. At the time of weighing, the moisture contents of barley lots to be malted were 11.6%, 11.6%, 8.9%, and 9.0%

for Oderbrucker, Wisconsin Barbless, Peatland, and Chevron, respectively. After steeping, the lots of barley were drained a uniform length of time, weighed, and placed in the germinator. The germinating barley was stirred twice daily, once after watering and 12 hours later. The placing of the different barley lots into the steep tank was scheduled in such a manner that a series containing 16 malts that had been germinated 2, 4, 6, and 8 days could be placed in the drier at the same time.

In Table I is given the percentage moisture for each variety after steeping to high or low moisture at 12° and 16°C. The time used for steeping at 16°C. was 24 and 60 hours for low and high moisture, respectively, for all varieties. Since the barley varieties were grown under three sets of conditions, it is probably only a coincidence that they absorbed water at approximately the same rate. The percentage for the four varieties averaged 42.7 and 48.8 at low and high moistures when steeped at 16°C. The time used in steeping at 12°C. was 34 and 72 hours, resulting in average moisture contents of 43.6% and 48.1%, respectively. In calculating moisture content out of steep, a loss of 2% and 2.5% in dry weight was estimated to have occurred during steeping for low and high moisture levels.

In these studies, an attempt was made to maintain moistures of about 43% and 49% throughout the growing period. Green malt moistures were slightly above these percentages. In order to maintain the moisture content as planned, it was necessary to anticipate respiration losses and to adjust weights accordingly. These losses were estimated on the basis of data obtained in a series involving 2 to 9 days of malting in the large chamber described by Dickson et al. (1938). This series, to be reported later, showed greater respiration losses for Oderbrucker than for Wisconsin Barbless. Little was known of the respiration rates of Peatland and Chevron and therefore estimates were readjusted after the first malting series was completed. To do this effectively, a schedule was outlined for the desired weight of the germinating barley on different days by compensating for loss in weight caused by respiration. Samples were weighed daily and brought to a prescribed weight. Different watering schedules were required for the different malting temperatures. The fact that the moisture content of the green malt agreed closely with the desired value demonstrates that the watering schedule was approximately correct.

The position of the cans in the drier was changed systematically during the period to minimize the influence of slight variations in temperature within the kiln. The temperatures and the times used in drying were approximately as follows: 25°C. at the beginning, which

was raised slowly to 35°C. and held for 24 hours; the temperature was then gradually raised to 45°C. and retained for 4 to 5 hours and finally the temperature was again increased to 55°C. with a finishing temperature of about 65°C. The total time for drying was 33 hours, after which treatment the moisture was near 6.8% as will be seen in Table I. This moisture content was perhaps higher than necessary; but there is evidence to show that higher moisture contents at the end of the drying schedule used are generally more likely to favor maximum expression of certain biochemical factors as will be shown in a later paper.

Physical and Chemical Analyses

The experimentally produced malts [3] were analyzed for the following physical and chemical factors: moisture content of steeped barley and green and dried malt, respiration and steep loss, root loss, recovery of malt from barley, growth of acrospire, kernel weight, extract, diastatic power (in degrees Lintner), malt nitrogen, wort nitrogen (reported as percentage of malt nitrogen), conversion time, and wort color.

Physical and chemical analyses already listed were made on the malts produced experimentally. Growth of acrospire was determined in the conventional manner and growth index calculated as follows:

Length of acrospire	Number kernels	Factor	Index
Dead	x	0	x
$0-\frac{1}{4}$	x	0.25	x
$\frac{1}{4}-\frac{1}{2}$	x	0.50	x
$\frac{1}{2}-\frac{3}{4}$	x	0.75	x
$\frac{3}{4}-1$	x	1.00	x
Overgrown	x	1.50	x
Total	x		x

The overgrown group has been arbitrarily multiplied by 1.5. It is realized that not all overgrown acrospires are 1.5 times the kernel length, but such a procedure will serve for making comparisons. Kernel weight on dry basis was determined as described by Shands (1937).

$$\frac{\text{Wt. barley dry basis} - \text{wt. uncleaned malt dry basis}}{\text{Wt. barley dry basis}} \times 100$$
$$= \text{respiration and steep loss in percent}$$

$$\frac{\text{Wt. uncleaned malt} - \text{wt. cleaned malt}}{\text{Wt. uncleaned malt}} \times 100 = \text{root loss in percent}$$

$$\frac{\text{Wt. cleaned malt dry basis}}{\text{Wt. barley dry basis}} \times 100 = \text{recovery in percent}$$

The chemical factors were determined by methods outlined by the American Society of Brewing Chemists. The data obtained for the malting-quality factors discussed have been tabulated for the 192 experimental malts. Some of the results will be presented in detail because this will afford opportunity for the reader to interpret results and form opinions independently of those discussed by the writers.

Respiration and Steep Loss

The method of conducting the experiment did not permit a ready separation of respiration and steep losses; therefore, they are combined in this paper. The detailed results of respiration and steep losses for the four varieties malted under different conditions are given in

TABLE II

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON PERCENT RESPIRATION AND STEEP LOSS COMBINED, OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	4.2	4.3	2.4	5.2	6.5	6.8	7.6	9.0
		Wis. Barbless	3.4	2.2	3.3	4.1	5.5	5.9	6.8	7.5
		Peatland	3.1	3.7	4.0	4.5	4.8	5.5	7.6	7.8
		Chevron	3.4	3.2	4.1	4.6	4.8	5.9	6.4	7.7
	16	Oderbrucker	4.2	5.6	5.8	8.0	6.5	9.4	8.7	10.7
		Wis. Barbless	3.7	4.0	5.0	6.7	5.5	7.9	7.3	8.1
		Peatland	3.3	4.0	4.7	6.7	5.5	8.1	7.0	8.8
		Chevron	2.9	4.4	4.5	6.4	5.8	8.1	7.1	9.5
	20	Oderbrucker	5.2	6.5	6.8	9.2	8.9	12.1	9.8	13.6
		Wis. Barbless	5.0	5.4	6.1	7.0	7.6	8.9	7.1	9.5
		Peatland	4.3	5.6	5.8	7.3	7.5	9.4	6.9	10.8
		Chevron	4.2	6.1	6.5	7.6	7.4	9.6	8.0	11.5
	24	Oderbrucker	5.5	7.5	7.7	11.4	10.2	16.2	13.3	16.0
		Wis. Barbless	4.5	6.0	5.5	7.9	8.0	10.8	9.5	13.1
		Peatland	4.1	6.1	6.6	7.7	8.1	11.1	9.4	16.1
		Chevron	4.4	6.9	6.7	9.2	8.3	11.5	9.2	12.3
12	12	Oderbrucker	4.7	5.2	5.0	5.8	6.4	7.6	7.4	9.5
		Wis. Barbless	4.1	4.3	4.4	4.8	5.3	6.4	6.4	7.9
	16	Oderbrucker	4.3	4.8	5.8	7.2	6.6	9.0	9.5	11.2
		Wis. Barbless	3.5	3.9	5.0	4.7	5.8	7.0	7.1	8.9
	20	Oderbrucker	5.5	6.4	7.0	9.0	8.6	11.5	11.6	15.8
		Wis. Barbless	4.4	5.0	5.8	6.2	6.1	8.9	9.2	12.0
	24	Oderbrucker	6.4	8.6	9.7	13.1	9.7	14.6	14.4	18.5
		Wis. Barbless	5.2	6.2	7.8	9.4	8.3	10.6	9.0	12.5

Table II. These losses ranged from about 3% in the shorter growth time to about 16% for high-moisture, high-temperature malts grown for the longer period of time. Losses increased with rise in malting temperature for all varieties. While the varieties used were not produced under strictly comparable conditions except for Peatland and Chevron, it is believed that varietal responses have been retained to such an extent that the reactions would change little had the varieties been produced under the same conditions. Even though the main purpose of this paper is to study the influence of such factors as growth time, temperature, and moisture, varietal responses must be carefully considered before conclusions are drawn. The varieties ranked in descending order for respiration and steep losses as follows: Oderbrucker, Peatland, Chevron, and Wisconsin Barbless. The differences between the average values of the respiration and steep losses of the respective varieties were statistically significant with the exception of that between Peatland and Chevron, indicating possible varietal differences with regard to these losses. Even though varieties were different in their average losses from steeping and respiration, it does not necessarily follow that each variety was different from the others under all sets of malting conditions. A summary of the F values from the analysis of variance for the several factors studied is given in Table XIII, where it will be seen that interaction values indicate that respiration and steep losses may be influenced by the particular choice of malting conditions. In the discussion of differences in malt quality as caused by variety, moisture, growth time, and malting temperature, as well as first or higher-order interactions, it is well to keep in mind that generalized statements might need modification if applied to a particular set of malting conditions.

Oderbrucker showed greater respiration and steep losses than the other three varieties in practically all cases. At the higher moisture, Wisconsin Barbless gave the lowest losses with Peatland and Chevron intermediate. The loss for Peatland was more between 6 and 8 days' growth than for the three other varieties but intermediate between 2 and 4 days' growth. High moisture caused greater daily losses than did lower moisture, the losses for 8 days at low moisture being approximately equal to estimated losses for 5 days at high moisture. There was little difference in the influence of 12° and 16°C. steep temperatures, the losses being slightly less for the latter temperature.

Root Loss

Root losses were influenced by malting conditions in approximately the same manner as respiration and steep losses, as can be seen from a comparison of Tables II and III. Much more root growth occurred

when either moisture or malting time increased.   Increase in malting temperature caused greater root losses with time increase, except for the longer growth periods at 20°C., where the rootlets tended to become brownish.   Chevron had greater losses than any other variety, while Peatland was second, with Oderbrucker and Wisconsin Barbless

TABLE III

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON PERCENT OF ROOT LOSS OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	.4	.8	1.7	2.0	2.7	3.7	3.5	4.6
		Wis. Barbless	.4	.4	1.7	2.0	2.6	3.5	3.2	4.0
		Peatland	.7	.9	2.0	2.5	2.7	3.8	3.3	4.8
		Chevron	.9	.9	2.1	3.0	2.7	4.5	3.2	5.3
	16	Oderbrucker	1.4	1.3	2.7	3.5	4.0	4.9	5.1	6.9
		Wis. Barbless	1.4	1.1	2.8	3.0	3.9	4.7	4.4	5.2
		Peatland	1.6	1.7	2.8	3.5	3.6	5.5	4.7	6.7
		Chevron	1.8	2.2	3.1	4.1	4.0	5.7	5.3	7.4
	20	Oderbrucker	1.6	2.9	3.2	4.8	3.8	7.3	4.2	6.5
		Wis. Barbless	1.6	2.9	3.6	4.7	4.1	6.0	4.2	6.0
		Peatland	1.8	4.7	3.2	5.5	4.0	7.6	3.9	7.0
		Chevron	2.0	4.0	3.5	6.1	4.3	7.5	4.2	7.3
	24	Oderbrucker	2.8	3.6	3.4	6.4	4.3	6.6	3.4	7.7
		Wis. Barbless	2.8	5.5	3.8	5.6	4.6	6.3	5.2	7.6
		Peatland	2.7	3.8	3.7	5.9	4.5	7.0	4.7	7.5
		Chevron	2.7	4.5	4.0	6.3	4.8	6.9	5.4	8.0
12	12	Oderbrucker	.3	.5	1.4	1.9	2.4	2.9	3.4	4.7
		Wis. Barbless	.2	.2	1.6	1.8	2.3	2.9	3.1	4.2
	16	Oderbrucker	1.0	1.1	2.3	3.7	3.5	5.2	3.8	6.0
		Wis. Barbless	1.0	.9	2.3	2.8	3.5	4.7	3.7	5.3
	20	Oderbrucker	1.8	2.8	3.9	6.3	4.8	6.3	5.7	8.0
		Wis. Barbless	2.0	2.6	4.2	5.7	4.4	6.1	5.1	7.8
	24	Oderbrucker	2.3	3.6	3.6	5.4	4.3	8.1	3.4	7.6
		Wis. Barbless	2.5	3.3	3.7	5.2	5.1	6.3	4.8	8.8

third and fourth respectively.   In the section of the experiment using two steep temperatures, it was found that malting temperatures, moisture, and time exercised important influences.   Oderbrucker root losses were not different from Wisconsin Barbless when a 12°C. steep temperature was used.   Regardless of steep temperature, root losses increased with increase in growth time except for the case already noted.

Recovery

The recovery of malt from barley, for each of the experimental maltings, can be considered as the complement of the additive effects of losses of steep, respiration, and rootlets. The data are given in Table IV and portrayed graphically in Figure 1a. That recovery

TABLE IV

The Influence of Temperature, Moisture, and Growth Time on Percent of Recovery of Four Barley Varieties Malted Experimentally

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	95.5	94.9	95.9	92.9	91.0	89.8	89.2	86.9
		Wis. Barbless	96.2	97.5	95.1	94.0	92.1	90.8	90.2	88.8
		Peatland	96.3	95.4	94.1	93.1	92.7	90.9	89.3	87.7
		Chevron	95.8	95.9	93.9	92.6	92.6	89.9	90.6	87.4
	16	Oderbrucker	94.4	93.2	91.6	88.8	89.8	86.2	86.6	83.1
		Wis. Barbless	94.9	95.0	92.4	90.6	90.9	87.7	88.6	87.1
		Peatland	95.2	94.4	92.6	90.0	91.1	86.9	88.6	84.9
		Chevron	95.4	93.5	92.6	89.8	90.4	86.7	88.0	83.9
	20	Oderbrucker	93.3	90.8	90.2	86.5	87.7	81.5	86.4	80.8
		Wis. Barbless	93.4	91.9	90.6	88.7	88.6	85.7	89.0	85.1
		Peatland	94.0	90.0	90.9	87.6	88.8	83.7	89.5	82.9
		Chevron	93.8	90.1	90.2	86.8	88.6	83.6	88.1	82.0
	24	Oderbrucker	91.8	89.2	89.1	82.9	85.9	78.3	83.3	77.6
		Wis. Barbless	92.9	90.7	90.9	86.9	87.8	83.6	85.8	80.3
		Peatland	93.3	90.3	89.9	86.9	87.8	82.7	86.4	77.6
		Chevron	93.0	88.9	89.6	85.1	87.3	82.4	85.9	80.7
12	12	Oderbrucker	95.0	94.4	93.6	92.4	91.3	89.7	89.4	86.3
		Wis. Barbless	95.8	95.5	94.1	93.4	92.5	90.9	90.7	88.2
	16	Oderbrucker	94.8	94.1	92.0	89.3	90.1	86.3	87.1	83.4
		Wis. Barbless	95.6	95.2	92.9	92.7	90.9	88.6	89.4	86.2
	20	Oderbrucker	93.2	91.0	89.4	95.3	87.1	82.9	83.3	77.5
		Wis. Barbless	93.7	92.5	90.3	88.5	89.0	85.6	86.2	81.1
	24	Oderbrucker	91.4	88.1	87.0	82.3	86.4	78.5	82.3	75.4
		Wis. Barbless	92.4	90.6	88.9	85.9	87.0	83.8	86.6	79.8

decreases with an increase in growth time in almost a straight-line relationship (2- to 6-day malting period) is shown by the curves in Figure 1a, which are based on the average recovery of the four varieties corresponding to the malting temperature and moisture level concerned. Recovery decreases with an increase of temperature and growth time at both moisture levels. Recovery also was less at the high moisture. Recovery varied from 96.2% to 77.6%, depending

upon variety and malting conditions.   Varieties responded somewhat differently to moisture, time, and temperature, the order of increasing recovery being as follows: Oderbrucker, Chevron, Peatland, and Wisconsin Barbless.   Oderbrucker recovery was lowest at each moisture, at each time interval, and at each temperature.   Differences between varieties were not as great at low moisture.

Steeping at 12°C. gave slightly less average recovery than at 16°C. Oderbrucker and Wisconsin Barbless reacted similarly to the two steep temperatures.   Recovery for 16°C. steep temperature was greater than expected in most cases for 8 days at malting temperatures of 20° and 24°C.   This may have unduly influenced the average for recovery at this steep temperature, thereby possibly invalidating the statement that recovery at 12°C. steep was less than that at 16°C.

TABLE V

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON GROWTH INDEX OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	49.5	48.3	50.3	56.3	56.0	92.0	68.0	95.5
		Wis. Barbless	49.8	49.8	53.3	68.8	71.5	96.3	77.0	105.8
		Peatland	49.3	50.3	52.8	58.3	75.3	93.0	73.5	99.0
		Chevron	49.3	49.3	53.0	69.0	73.3	83.0	71.5	98.3
	16	Oderbrucker	50.8	50.8	65.5	89.8	70.5	102.8	79.3	106.5
		Wis. Barbless	49.8	51.3	62.0	95.5	74.0	98.8	79.0	102.8
		Peatland	50.8	53.0	65.3	87.3	68.8	94.3	78.3	103.8
		Chevron	49.0	53.0	56.5	86.3	69.8	94.3	78.8	95.3
	20	Oderbrucker	49.3	69.3	58.3	81.8	69.8	90.3	68.0	96.8
		Wis. Barbless	49.5	60.5	62.5	91.3	68.0	98.0	76.5	109.0
		Peatland	49.3	65.0	67.3	88.0	72.0	87.3	74.3	97.0
		Chevron	46.3	61.5	60.5	84.8	71.3	86.5	74.5	102.8
	24	Oderbrucker	45.0	76.0	56.5	83.0	57.0	95.3	69.3	89.0
		Wis. Barbless	52.5	75.0	61.3	80.8	64.8	98.8	75.8	95.3
		Peatland	53.8	63.0	57.5	78.5	57.5	80.5	67.0	106.0
		Chevron	53.5	73.0	57.0	76.8	63.5	82.5	75.5	79.0
12	12	Oderbrucker	48.8	49.0	49.0	64.8	65.8	91.3	73.0	100.0
		Wis. Barbless	49.5	50.5	51.8	68.3	74.8	100.0	77.8	107.8
	16	Oderbrucker	49.8	49.8	59.3	93.0	82.3	98.0	77.3	106.3
		Wis. Barbless	49.3	49.8	66.0	91.5	73.0	97.3	86.8	112.3
	20	Oderbrucker	51.3	65.0	65.3	94.3	70.8	102.8	71.5	105.0
		Wis. Barbless	50.5	62.8	67.8	92.8	72.3	99.8	79.3	105.5
	24	Oderbrucker	50.3	81.0	68.0	91.3	57.8	89.8	79.5	96.5
		Wis. Barbless	51.8	69.3	65.0	100.8	77.0	90.5	75.0	90.0

Growth Index

Growth index values for the malts produced experimentally are recorded in Table V. Moisture exercised considerable influence, with the high moisture giving much more growth in all instances, the magnitude of the difference generally increasing with number of days. Average growth index showed a maximum at 16°C. After 8 days, however, the average of growth at 12°C. was nearly as much as at 16°C. or 20°C. and more than at 24°C. Growths for 12°C. and 16°C. malts at 2 days, at high moisture, were both low; by the end of the fourth day the average growth at 16°C. was greater than at 20°C. or 24°C. and by the end of the sixth day the 12°C. malts had grown as much as the 20°C. or 24°C. malts. The final growth index at 8 days

TABLE VI

The Influence of Temperature, Moisture, and Growth Time on Kernel Weight of Malt (mg.) of Four Barley Varieties Malted Experimentally

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	27.1	26.6	27.2	26.0	25.8	25.6	25.6	24.8
		Wis. Barbless	25.3	25.7	24.5	24.5	23.5	23.7	23.5	23.0
		Peatland	24.6	23.8	23.8	23.9	24.0	23.3	22.2	22.2
		Chevron	24.0	24.1	23.4	22.9	23.0	21.5	22.5	23.9
	16	Oderbrucker	27.0	26.3	26.0	25.7	25.0	24.0	24.5	23.2
		Wis. Barbless	25.0	24.8	23.6	23.5	23.6	22.5	22.8	22.7
		Peatland	24.3	23.3	24.1·	22.7	23.8	21.6	22.6	21.9
		Chevron	24.2	23.9	23.3	22.8	22.5	21.7	21.6	21.1
	20	Oderbrucker	26.2	25.2	25.5	24.6	24.3	23.3	25.0	22.9
		Wis. Barbless	24.0	23.4	23.8	22.9	22.9	21.1	23.0	22.7
		Peatland	24.4	23.3	23.1	22.7	22.4	21.0	22.7	21.6
		Chevron	23.7	23.2	22.7	21.9	21.9	21.3	22.2	20.1
	24	Oderbrucker	25.9	24.8	25.6	20.4	24.5	21.6	23.6	22.3
		Wis. Barbless	24.2	23.9	23.7	22.5	22.9	21.5	22.3	21.3
		Peatland	23.8	22.9	22.5	22.3	21.7	21.9	21.5	20.0
		Chevron	23.4	22.5	21.2	21.7	21.8	22.9	21.6	20.3
12	12	Oderbrucker	26.7	26.4	26.9	26.0	25.2	25.3	25.4	24.3
		Wis. Barbless	25.0	24.4	23.8	24.1	23.1	23.1	24.3	22.2
	16	Oderbrucker	27.2	26.6	25.7	25.2	25.9	24.1	24.5	26.7
		Wis. Barbless	24.6	25.1	24.4	23.4	22.9	22.8	23.4	22.0
	20	Oderbrucker	27.2	25.6	25.6	24.0	24.8	23.8	23.7	22.0
		Wis. Barbless	23.9	24.3	24.2	22.2	23.6	21.7	22.6	21.0
	24	Oderbrucker	25.6	24.4	24.2	23.1	24.8	22.6	23.0	21.1
		Wis. Barbless	24.2	23.5	22.9	21.7	22.0	21.7	22.6	20.4

at low moisture was less than 80 for all malting temperatures, indicating that the low moisture may have been a limiting factor. The varieties were not greatly different in growth indices when steeped at 16°C., but average indices at both steep temperatures show that Wisconsin Barbless produced more growth than Oderbrucker.

Kernel Weight

Kernel weight of malt, dry basis, followed the same general trends as recovery, which can be seen by comparing Tables IV and VI. High moisture reduced the kernel weight for the average of all varieties as did increase in growth time and temperature. At both moisture levels, kernel weight was reduced with increase in malting temperature. The varieties ranked in decreasing order for kernel weight of malt, as follows: Oderbrucker, Wisconsin Barbless, Peatland, and Chevron. Kernel weight of malt did not seem to be influenced greatly by steep temperatures although the same effect of moisture and temperature as indicated above was evident. Apparently the number of kernels used in determining kernel weight was not large enough or conditions were not controlled closely enough, since certain malting procedures gave weights that varied from the expected. For example the weights at 8 days for malts from 20° malting and 16° steep were higher than the weight for 6 days.

Extract

Moisture during malting proved to be important in influencing extract content, as shown in Table VII and Figure 1b. The extract values for 2-day malts at the lower temperatures are probably not reliable, since there was insufficient enzyme liberated to convert all of the starch in one hour. This is substantiated by the conversion times given in Table XI. Greatest variation in extract content occurred at the malting temperature of 12°C., where Wisconsin Barbless malted at high moisture for 2 days gave 59.7% and Oderbrucker malted at low moisture for 8 days gave 77.8%. Whether this figure is the highest obtainable is problematical, but it appears to be very near the maximum available in the barley. The average of high- and low-moisture 2-day malts showed an ascending extract with temperature in the order of 12°, 16°, 24°, and 20°C. The higher value for the 20°C. malts possibly indicates that 24°C. was too high for the best expression of extract in a 2-day malt. At 4 days, extract values for different moistures and temperatures converged within a 2% range and spread for later days to a maximum difference of about 5% at 8 days. After 8 days, using an average of the 2 moistures and all varieties, it will be seen that 12°C. gave distinctly the highest extract,

followed in order by 16°, 20°, and 24°C. Extract was gradually reduced after the 2-day period with increased malting times for 20° and 24°C. malts at the high moisture. Even with low moisture, extract was reduced increasingly after the 2 days' malting at 24°C. Maximum expression of extract was found for 16°, 20°, and 24°C.,

TABLE VII

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON PERCENT OF MALT EXTRACT OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	67.2	64.9	75.1	73.1	77.0	75.3	77.8	76.2
		Wis. Barbless	67.3	59.7	71.1	70.0	72.2	71.4	72.5	72.3
		Peatland	67.6	67.2	74.0	73.6	74.9	74.0	76.6	74.8
		Chevron	68.5	66.9	72.8	72.9	73.8	73.5	74.8	73.7
	16	Oderbrucker	72.5	71.5	75.8	75.9	75.4	75.7	76.1	74.6
		Wis. Barbless	69.6	69.6	71.5	71.6	70.8	71.4	71.1	69.9
		Peatland	69.4	71.8	74.0	74.8	74.2	74.5	74.3	73.5
		Chevron	71.4	71.6	72.9	72.8	73.1	72.8	72.5	72.5
	20	Oderbrucker	75.1	75.2	76.2	75.3	76.5	75.1	75.8	74.2
		Wis. Barbless	71.7	71.3	71.3	71.6	71.6	71.2	71.0	70.0
		Peatland	73.6	74.6	74.0	73.5	74.6	73.5	73.0	72.6
		Chevron	73.1	74.1	73.5	72.7	73.2	72.2	72.5	71.8
	24	Oderbrucker	75.6	76.0	74.1	74.5	74.0	72.0	72.8	71.9
		Wis. Barbless	70.2	71.7	70.6	71.0	70.5	71.1	70.7	70.4
		Peatland	72.7	74.0	73.4	72.3	73.3	72.8	72.8	72.7
		Chevron	71.3	73.1	71.3	72.0	72.4	71.3	72.0	70.5
12	12	Oderbrucker	66.0	67.9	74.8	74.9	77.0	76.1	77.7	77.1
		Wis. Barbless	66.4	61.7	71.2	70.9	72.0	71.8	72.2	72.5
	16	Oderbrucker	72.3	71.6	76.1	75.6	76.4	75.1	77.7	75.0
		Wis. Barbless	69.6	68.5	71.8	71.6	70.7	70.7	70.5	70.4
	20	Oderbrucker	75.5	75.9	75.9	75.1	77.0	75.6	76.5	75.0
		Wis. Barbless	71.2	71.0	70.9	71.3	71.1	71.2	71.9	71.3
	24	Oderbrucker	76.2	76.9	76.7	74.7	73.2	73.1	71.8	70.8
		Wis. Barbless	71.1	72.1	71.7	71.7	70.3	69.8	69.9	69.7

but not definitely for 12°C. The varietal order for extract from high to low was Oderbrucker, Peatland, Chevron, and Wisconsin Barbless and the differences were significant. No great difference in varietal response was encountered for moistures, times of malting, or temperatures of malting. Extract of Oderbrucker decreased more than that of Wisconsin Barbless for longer growth times at 24°C. This is probably not a varietal difference, since Oderbrucker was attacked by

molds. At 16°C., low moisture gave less extract at 2 days than the high moisture, but this might have been caused by a greater production of diastatic enzymes at the higher moisture. Average extract content for Oderbrucker was about the same for the 12° and 16°C. steep temperatures. The same can be said of Wisconsin Barbless.

Diastatic Power

The range in diastatic power varied from 52° to 260° Lintner, according to variety and treatment. (See Table VIII and Figure 1c.) At the outset it may be said that Oderbrucker did not respond as well to malting conditions as expected. This belief is supported by some unpublished experimental malting studies. Moisture, temperature of

TABLE VIII

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON DIASTASE (°L.) OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	62	67	95	99	107	132	150	156
		Wis. Barbless	65	60	101	116	124	134	143	167
		Peatland	52	88	111	132	160	185	154	177
		Chevron	83	57	163	189	206	258	231	260
	16	Oderbrucker	73	76	97	96	117	115	111	128
		Wis. Barbless	78	95	121	109	141	131	121	164
		Peatland	72	96	106	136	138	192	144	197
		Chevron	116	128	195	167	203	242	198	257
	20	Oderbrucker	77	97	114	123	124	146	89	133
		Wis. Barbless	78	97	109	137	96	153	126	167
		Peatland	95	122	105	128	126	172	121	140
		Chevron	135	162	149	223	176	219	176	189
	24	Oderbrucker	73	100	105	124	88	121	93	115
		Wis. Barbless	80	110	80	143	98	126	106	140
		Peatland	74	131	70	144	85	153	87	145
		Chevron	104	154	78	161	120	168	132	202
12	12	Oderbrucker	63	71	98	104	115	121	146	136
		Wis. Barbless	66	60	106	111	126	136	155	169
	16	Oderbrucker	79	89	114	122	130	129	121	155
		Wis. Barbless	89	79	119	135	117	147	110	161
	20	Oderbrucker	96	126	95	145	121	118	98	133
		Wis. Barbless	94	119	124	133	105	138	103	145
	24	Oderbrucker	98	110	103	124	105	119	83	121
		Wis. Barbless	107	132	107	143	93	147	98	145

malting, and length of growing time had highly important influences on diastase. Under the given conditions there seemed to be a close relationship between the factors that influenced acrospire growth and the development of diastatic power. High moisture favored the development of diastase. Diastase generally increased with malting-time increase, with marked exceptions after the 6-day period. Using the averages of all malting temperatures, diastatic power increased at each moisture until the sixth day, when a break occurred. At high moisture the increase in diastatic power from 6 to 8 days was slight, while at the low moisture there was no increase with an increase in time. The increase of diastatic power with time at high moisture was rather rapid at 12° and 16°C. through 8 days. At 24°C. diastase gradually increased to 8 days, but at 20°C. it declined after 6 days. At 2 days' growth, diastase was increased with increase in temperature, but as time increased the low-temperature malts increased in diastase faster than high-temperature malts. The varieties ranked on the basis of diastatic power from high to low in the following order: Chevron, Peatland, Wisconsin Barbless, and Oderbrucker. The highest diastatic powers for the varieties were 260, 197, 167, and 156, in the order named. As suggested earlier, Oderbrucker was distinctly off type from its usual reaction. Peatland averaged 40°L. more diastase at high moisture than at low moisture, while Oderbrucker had only 16°L. more, which is evidence of unequal varietal response to moisture increase. High temperatures depressed the diastatic power of Chevron and Peatland relatively more than of Oderbrucker or Wisconsin Barbless. Using both moistures and all temperatures, Chevron increased 88°L. between 2 and 8 days of malting, while Peatland and Wisconsin Barbless increased 54° and 59°L., respectively, during that period. This suggests that the rate of enzymatic activation is different for Peatland and Chevron. Steeping and malting temperatures used in this experiment did not seem to have clear-cut influences on the lots of Oderbrucker or Wisconsin Barbless barley. In Oderbrucker and Wisconsin Barbless, as was true with the other two varieties, 12° and 16°C. malting temperatures developed less diastase in the early malting periods than the higher temperatures 20° and 24°C., but the reverse was true at the end of the malting period.

The 12°C. steep gave higher diastatic values for early growth periods than 16°C. steep, but this influence did not continue throughout the 8 days' growth period.

Malt Nitrogen

The influence of growth time and malting temperature on malt nitrogen was not so clear-cut as on other factors studied. Moisture seemed to have little effect except at 8 days' growth, where high mois-

ture gave higher nitrogen on a percentage basis. (See Table IX.) At this same time respiration loss was greatest with recovery being least, and therefore malt nitrogen made up a higher percentage of the total dry matter than in the earlier stage of malting. One may speculate that, during growth, carbohydrates and nitrogen compounds had both

TABLE IX

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON PERCENT OF MALT NITROGEN OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	1.96	2.18	2.06	2.04	1.97	2.05	1.98	2.07
		Wis. Barbless	2.19	2.30	2.11	2.11	2.05	2.08	2.13	2.10
		Peatland	2.27	2.48	2.27	2.37	2.24	2.27	2.22	2.37
		Chevron	2.50	2.24	2.50	2.60	2.50	2.35	2.47	2.54
	16	Oderbrucker	2.23	2.06	2.04	2.03	2.04	1.96	2.04	2.23
		Wis. Barbless	2.27	2.23	2.07	2.11	2.12	2.10	2.13	2.26
		Peatland	2.39	2.35	2.49	2.34	2.30	2.46	2.30	2.50
		Chevron	2.59	2.54	2.60	2.54	2.54	2.45	2.55	2.56
	20	Oderbrucker	2.00	2.00	2.07	2.10	2.06	2.07	2.12	2.13
		Wis. Barbless	2.12	2.12	2.14	2.05	2.08	2.05	2.21	2.15
		Peatland	2.34	2.26	2.34	2.31	2.32	2.34	2.37	2.41
		Chevron	2.53	2.47	2.52	2.49	2.54	2.55	2.54	2.61
	24	Oderbrucker	2.05	2.05	2.07	2.02	2.14	2.14	2.11	2.17
		Wis. Barbless	2.09	2.18	2.07	2.08	2.10	2.18	2.16	2.23
		Peatland	2.34	2.37	2.28	2.34	2.37	2.39	2.32	2.40
		Chevron	2.56	2.42	2.58	2.66	2.54	2.54	2.66	2.65
12	12	Oderbrucker	2.14	2.08	1.98	1.98	1.98	2.03	2.03	2.02
		Wis. Barbless	2.21	2.13	2.01	2.05	2.02	2.07	2.07	2.09
	16	Oderbrucker	2.06	2.04	2.09	2.05	2.13	2.05	2.08	2.16
		Wis. Barbless	2.23	2.16	2.13	2.16	2.13	2.13	2.19	2.18
	20	Oderbrucker	2.02	2.01	1.98	2.02	2.12	2.07	2.09	2.12
		Wis. Barbless	2.14	2.08	2.13	2.07	2.10	2.14	2.13	2.11
	24	Oderbrucker	2.05	2.08	2.06	2.09	2.11	2.13	2.13	2.17
		Wis. Barbless	2.11	2.05	2.10	2.10	2.18	2.05	2.16	2.18

been respired, but the proportion of carbohydrates combusted was much greater than of nitrogen. The nitrogen translocated to rootlets removed before analysis should also be kept in mind in discussing the nitrogen metabolism (degradation). The nitrogen trend on a percentage of dry matter was downward for the first 6 days and then moved upward on the eighth day. At 4 days the nitrogen percentage at each

malting temperature was about the same, whereas at 8 days the values lined up in the following descending order: 24°, 20°, and 16°, all of which are about equal, and 12°C. the lowest. For low moisture at 8 days there was some tendency for the malt nitrogen to increase with temperature increase. Nitrogen in Wisconsin Barbless was consistently less at 4 and 6 days than at 2 and 8 days: Chevron and Oderbrucker increased in most cases from 6 to 8 days, while Peatland did not change greatly throughout the growing period. Chevron had slightly more nitrogen at the lower moisture, while the other varieties in most cases had more at the higher moisture. The varietal rank concerning malt nitrogen was the same as that for diastatic power and root loss, with Chevron being first. Steep temperature apparently had little effect on the malt nitrogen of Oderbrucker and Wisconsin Barbless.

TABLE X

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON WORT
NITROGEN IN RELATION TO MALT NITROGEN OF FOUR BARLEY
VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	18.3	15.6	29.1	26.2	34.2	31.5	37.9	39.1
		Wis. Barbless	17.5	15.2	24.5	24.2	25.5	28.2	26.7	31.4
		Peatland	20.6	19.0	26.2	26.4	31.5	31.9	34.1	34.9
		Chevron	21.1	20.0	29.4	28.4	31.6	37.3	33.7	37.0
	16	Oderbrucker	23.4	23.5	32.7	35.4	34.6	41.1	37.6	38.4
		Wis. Barbless	19.8	19.9	26.1	27.9	25.1	29.2	26.6	29.5
		Peatland	23.6	24.6	27.2	32.8	30.5	32.9	31.7	33.6
		Chevron	24.4	26.6	27.9	32.3	28.4	35.2	30.0	35.5
	20	Oderbrucker	26.7	30.2	31.4	34.2	33.5	38.7	35.4	39.3
		Wis. Barbless	23.6	26.3	23.9	27.5	24.3	28.5	23.3	26.3
		Peatland	26.7	33.0	26.9	31.0	28.2	32.5	27.2	32.2
		Chevron	26.5	32.9	27.3	30.9	26.7	31.3	27.1	30.9
	24	Oderbrucker	29.3	33.7	41.8	40.7	28.9	38.6	32.1	37.0
		Wis. Barbless	24.0	27.4	24.5	28.4	24.3	30.2	25.4	28.2
		Peatland	26.8	30.9	26.9	30.4	28.0	32.5	30.7	39.9
		Chevron	24.3	31.0	25.2	30.0	27.7	32.0	27.5	30.7
12	12	Oderbrucker	17.3	18.3	28.4	29.5	35.1	35.7	37.4	42.8
		Wis. Barbless	17.7	17.3	26.6	25.9	28.6	29.9	28.6	32.5
	16	Oderbrucker	24.2	22.6	31.0	35.6	32.6	38.7	37.4	40.6
		Wis. Barbless	19.4	27.7	25.7	27.5	25.8	28.7	25.0	30.0
	20	Oderbrucker	28.2	30.7	33.7	41.1	34.4	39.6	36.5	45.8
		Wis. Barbless	23.3	26.9	25.6	30.0	24.7	33.8	25.0	28.9
	24	Oderbrucker	28.6	33.5	34.9	41.3	28.6	38.5	33.1	38.1
		Wis. Barbless	23.7	28.7	25.0	28.3	24.0	29.3	24.9	31.1

Wort Nitrogen as Percentage of Malt Nitrogen

The percentage of wort nitrogen in relation to malt nitrogen ranged from more than 45% to about 15% according to variety and method of malting. The data are given in Table X and curves in Figure 1d, where it will be seen that the trends closely parallel those for diastatic power. For the most part the influence of malting environment was pronounced. With moisture alone considered and temperatures averaged, the higher moisture gave a higher percentage of wort nitrogen at all growth periods and it increased with longer malting time for either moisture. Malting temperature also influenced soluble nitrogen percentage, especially when time of growth was considered, because the high temperatures favored more soluble nitrogen in early growth phases while the lower temperatures were more favorable at the longer growth time. At 6 and 8 days' growth, wort nitrogen percentage was greater at the lower temperatures. There was less difference between the wort nitrogen percentages of 6- and 8-day malts than of 2- and 4-day malts. There was a similarity between wort nitrogen trends at both moisture levels as growth time increased. Using averages of all malt times and both moistures, the wort nitrogen of Chevron malts decreased with temperature rise, which is in contrast to the other three varieties. All varieties showed an increased wort nitrogen percentage with increase in growing time, but the increase of Chevron and Wisconsin Barbless between 6 and 8 days was smaller than for the other two varieties. The varietal rank from high to low for percentage wort nitrogen was Oderbrucker, Peatland, Chevron, and Wisconsin Barbless, with the second and third varieties not being significantly different. Steep temperature seemed to influence soluble nitrogen, since malts from the 12°C. steep gave higher percentages than did those from the 16°C. steep.

Conversion Time

Conversion time given in Table XI varied from more than an hour to less than 5 minutes, according to malting treatment and variety. For the most part conversion time decreased as malting time increased. The effect of malting temperature on conversion time was much more important during the earlier growth period than later. The conversion time for 12°C. and 16°C. malting temperature was much greater at 2 days than 20° or 24°C., while at 8 days the 12° and 16°C. malt temperatures averaged about the same conversion as 20° and 24°C. The high-moisture malts grown at 12°C. for 4 days were slower in conversion time than the low-moisture malts. Otherwise low-moisture malts required more time for conversion on the average than did high-moisture malts. Conversion time at high moisture content

decreased in general with malting time increase, but at low moisture it increased at 8 days over that for 6 or 4 days. The varietal rank from low to high for conversion time was Chevron, Oderbrucker, Wisconsin Barbless, and Peatland, with no significant difference between the middle pair. This arrangement is considerably different from the

TABLE XI

The Influence of Temperature, Moisture, and Growth Time on Conversion Time in Minutes of Four Barley Varieties Malted Experimentally

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	>60	>60	15	35	10–15	10	25–30	7–10
		Wis. Barbless	>60	>60	20	30	10–15	10–15	10	5–7
		Peatland	>60	>60	15	25	10	10–15	10–15	10
		Chevron	>60	>60	7	10	7	7	7–10	7
	16	Oderbrucker	60	>60	7	5	5–7	7	5–7	<5
		Wis. Barbless	35	30–35	15	10	15	10	15–20	10
		Peatland	45	30–35	15	10	15	10	15–20	10
		Chevron	30	20	7	7–10	10	5	7	7
	20	Oderbrucker	20	10–15	7	7–10	5	<5	10	5–7
		Wis. Barbless	20–25	10–15	15	10–15	20	10–15	20	5–7
		Peatland	30	10–15	10–15	10	30	7–10	20	15
		Chevron	20	10	10	7–10	15	7–10	15	10
	24	Oderbrucker	10–15	10–15	10–15	15–20	15	5–7	7	<5
		Wis. Barbless	20	10–15	25	10–15	20	10–15	10	10–15
		Peatland	50	15–20	35	15–20	25	10–15	20	10–15
		Chevron	15	15–20	15	15	15	<5	10	5
12	12	Oderbrucker	>60	>60	20	20	7	6	<5	5–7
		Wis. Barbless	>60	>60	20	15	10	7–10	10–15	10
	16	Oderbrucker	>60	>60	10	15	5	<5	7	5–7
		Wis. Barbless	>60	>60	20	15	15–20	5–7	10	10
	20	Oderbrucker	10–15	15	7–10	5	5	<5	10	5
		Wis. Barbless	15	10–15	15	10	15	7	15–20	10–15
	24	Oderbrucker	10	7	10	5–7	10–15	<5	7	<5
		Wis. Barbless	20	10	15	7	20–25	7–10	10	7–10

varietal rank when considering nitrogen in the wort, or diastase. It is interesting to note that Wisconsin Barbless required more than 5 minutes for all of the methods of malting. Peatland grown at low moisture required more time for conversion than did other varieties. Chevron malt grown at 16°C. converted most rapidly, while the malts from the other varieties were more rapid at the 20°C. malting temperature. At high moisture Oderbrucker and Wisconsin Barbless required

the least time for conversion at 8 days, while Chevron and Peatland required the least time at 6 days. Conversion time of Oderbrucker and Wisconsin Barbless malts was a little less for malts steeped at 12°C. than for those steeped at 16°C.

TABLE XII

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND GROWTH TIME ON WORT COLOR (LOV. 52) OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY

Steep temp., °C.	Malting temp., °C.	Variety of barley	Days of growth and moisture							
			2		4		6		8	
			Low	High	Low	High	Low	High	Low	High
16	12	Oderbrucker	1.4	1.3	1.6	1.4	1.4	1.4	1.5	1.5
		Wis. Barbless	1.4	1.0	1.5	1.3	1.5	1.3	1.4	1.4
		Peatland	1.1	1.1	1.4	1.4	1.5	1.4	1.5	1.4
		Chevron	1.2	1.3	1.5	1.4	1.8	1.5	1.5	1.6
	16	Oderbrucker	1.4	1.4	1.4	1.4	1.5	1.6	1.6	1.8
		Wis. Barbless	1.4	1.4	1.5	1.4	1.5	1.5	1.4	1.4
		Peatland	1.4	1.4	1.5	1.6	1.5	1.4	1.4	1.5
		Chevron	1.4	1.3	1.5	1.5	1.5	1.4	1.5	1.5
	20	Oderbrucker	1.6	1.4	1.4	1.6	1.5	1.8	2.0	1.6
		Wis. Barbless	1.5	1.4	1.5	1.5	1.5	1.6	2.0	1.5
		Peatland	1.4	1.8	1.6	1.7	1.5	1.6	1.5	1.6
		Chevron	1.4	1.4	1.6	1.4	1.5	1.6	1.5	1.6
	24	Oderbrucker	1.8	1.4	1.4	1.9	1.5	1.2	1.9	2.1
		Wis. Barbless	1.9	1.5	1.6	1.8	1.9	1.9	2.0	2.8
		Peatland	1.8	1.6	1.8	2.0		2.0	2.3	2.3
		Chevron	1.8	1.5	1.9	1.9	1.8	1.9	1.9	1.6
12	12	Oderbrucker	1.1	1.2	1.5	1.4	1.3	1.3	1.4	1.5
		Wis. Barbless	1.1	1.0	1.4	1.4	1.3	1.3	1.3	1.4
	16	Oderbrucker	1.4	1.4	1.3	1.4	1.4	1.5	1.6	1.8
		Wis. Barbless	1.1	1.1	1.4	1.3	1.4	1.4	1.4	1.4
	20	Oderbrucker	1.3	1.3	1.5	1.6	1.3	1.4	1.4	1.5
		Wis. Barbless	1.4	1.3	1.5	1.4	1.6	1.4	1.4	1.4
	24	Oderbrucker	1.4	1.5	1.5	1.6	1.5	1.9	2.5	2.5
		Wis. Barbless	1.5	1.3	1.5	1.4	1.6	1.4	1.9	2.0

Color

Color of wort in Lovibond units (Series 52) varied from 1.0 to 2.8 as recorded in Table XII. Color increased with the longer growth period and also with higher malting temperatures. Oderbrucker malts did not increase in color at the 24° malting temperature as much as the other varieties. Moisture of malting as well as variety had little influence on color. In the portion of the experiment using

Oderbrucker and Wisconsin Barbless at 12° and 16°C. steeps, malting temperature again was important, the higher temperatures giving higher color reactions, and also color increased with longer growth period.   Lower steep temperatures gave less color.   There was an increase in color between 2 and 4 days' growth, but increases in color were greatest at 8 days with higher temperatures.   Oderbrucker and Wisconsin Barbless had the same color reading at the 16° steep but the color of the latter was less in the 12°C. steep.   As an average for both steep temperatures, the two varieties had nearly the same color for 4 and 6 days' growth, but Wisconsin Barbless had slightly less color than Oderbrucker at 2 and 8 days' growth.   Wisconsin Barbless averaged less color at high moisture than Oderbrucker, when both steep temperatures are considered.

Analysis of Variance

The data in Tables II to XII, inclusive, have been subjected to the analysis of variance, recognizing, however, limitations in the validity of the procedure for interpretation of the results.   In Table XIII

TABLE XIII

SUMMARY OF F VALUES FROM ANALYSIS OF VARIANCE COMPARED TO 1% AND 5% LEVELS OF SIGNIFICANCE OF FOUR BARLEY VARIETIES MALTED EXPERIMENTALLY AT 16°C. STEEP TEMPERATURE

	DF	Respiration and steep loss	Root loss	Recovery	Growth index	Kernel wt. of malt	Extract of malt	Diastatic power of malt	Malt nitrogen	Wort N in relation to malt N	Conversion time	Wort color
Varieties (V)	3	1[1]	1	1	—	1	1	1	1	1	1	—
Moisture (M)	1	1	1	1	1	1	1	1	—	1	1	—
Days Growth (D)	3	1	1	1	1	1	1	1	1	1	1	1
Malt Temperature (MT)	3	1	1	1	1	1	1	1	1	1	1	1
V×M	3	5[2]	1	—	—	5	—	1	5	—	1	—
V×D	9	5	—	5	—	—	—	1	1	1	5	—
V×MT	9	5	5	5	—	—	—	1	—	1	1	5
M×D	3	1	1	1	1	—	—	5	5	5	5	—
M×MT	3	1	1	1	—	5	1	1	—	1	1	—
D×MT	3	1	1	1	1	—	1	1	1	1	1	—
V×M×D	9	—	—	—	—	—	—	—	—	—	—	—
V×D×MT	27	—	—	—	—	—	5	1	1	1	1	—
M×D×MT	9	—	—	—	5	—	—	1	5	—	—	—
V×M×MT	9	—	5	—	—	—	—	—	—	—	—	—
V×M×D×MT	27	—	—	—	—	—	—	—	—	—	—	—

[1] 1 = excess of 1% level of significance.
[2] 5 = excess of 5% level of significance.

is given a summary of the F values obtained in the section of the study using the 4 varieties, 2 moistures, 4 growth periods, and 4 malt temperatures.   The figure "1" denotes that the F value exceeds the requirement of the 1% point, and "5" indicates that the F value obtained was in excess of the amount required for the 5% level of significance.   Where "−" occurs the F value was insignificant.   Part of the foregoing

discussion has been based on this summary. Nevertheless a brief review of the analysis might be of benefit at this point.

Varieties proved different for all malting factors studied except for growth index and color of wort. This does not mean that all varieties were different from each of the remaining three, but that at least one variety was different from one or more of the others. High and low malting moistures had different effects on all factors observed except for malt nitrogen and wort color. Growth time (2, 4, 6, and 8 days) and malting temperature influenced sharply all malting factors studied in this investigation. Varieties and moistures in this study did not interact for extract, wort nitrogen, growth index, and color of wort, but responded differentially to other factors mentioned in this study. Varieties and malting temperatures interacted similarly to varieties and days' growth except for root loss, malt nitrogen, and wort color. These first-order interactions were significant for growth index, kernel weight, and extract. Moisture and growth time influenced differently all factors tested except extract, kernel weight, and wort color. Differential reactions were recorded for moisture and malt temperatures for all malting measurements except malt nitrogen, growth index, and color of wort. And finally, as might be expected, growth time and malting temperature influenced all malting factors differentially except for kernel weight and color of wort. To review the analysis of variance, some of the interactions that proved statistically significant may be indicative of natural trends that have been produced by a series of temperatures or growth periods, and in this light the trends are what might be expected instead of random or disorderly responses. Where insignificant interactions occur as in the case of extract, there is evidence that an optimum has been reached with lower points on either side. In this analysis the third-order interaction of varieties × moisture × growth periods × malting temperatures, making up 27 degrees of freedom, was used as error.

In the portion of the experiment concerning the two steep temperatures, the results have been interpreted cautiously, because the Oderbrucker barley did not react as anticipated in the light of other experimental malting. Only the action of steep temperature and the interactions will be discussed. Steep temperature (12° and 16°C.) affected recovery, growth, wort nitrogen, and wort color. Varieties and steep temperature interacted only for wort color. Moisture and steep temperature reacted differentially for wort nitrogen and color. Time of growth and steep temperature influenced root loss, recovery, growth, and wort color unequally. And finally moisture and steep temperature interacted for root loss, recovery, and conversion time. Even though the differences attributable to steep temperatures are not

great it is believed on the basis of unpublished work that a wider range in steep temperatures would prove to have more influence on the quality of the finished malt.

Discussion and Summary

Recovery of malt (the proportion of cleaned malt to the amount of barley) is influenced markedly by moisture, malting temperature, and growth time.   Since recovery is determined largely by root loss, and also by respiration and steep loss, these three factors bear a close relationship.   Recovery for practical purposes may be considered as the complement of the combination of the three losses just named. Likewise a comparison of the kernel weight of malt with the kernel weight of barley should measure recovery rather accurately.   Since these measurements are closely associated, there is a general similarity of their curves and trends as produced by different malting conditions. A similarity, though not to such an extent, exists between growth index, diastase, and wort nitrogen.   What influences one seems likely to influence the other.

It should be emphasized here that this association of malt factors holds only for the given set of conditions studied and perhaps should not be interpreted too broadly.   Extract, malt nitrogen, color, and conversion time seem to be independent in their responses to the different malting conditions used.   From the foregoing data the writers would like to point out that factors concerned with malting quality are greatly influenced by conditions that may be controlled under malt house operation.   It is granted that some of the methods used experimentally are not practicable for a commercial malt house, but this type of experimentation is necessary in order to learn trends and effects.   These responses are greater than those described in a recent paper by Sallans and Anderson (1939), probably because a wider range of conditions was used.

Finally, it should be pointed out that this study indicates that no simple method has been found that will produce maxima for all quality and efficiency factors.   For example high extract may be obtained by using a low moisture, low temperature, and long malting time.   This, however, does not produce the greatest wort nitrogen, diastase or shortest conversion time.   On the contrary, high diastase may be produced by malting at high moisture for a longer period at a low temperature, but this does not give the greatest extract.   Or, if only a short period of time is allowed for malting, temperature and moisture increases would serve to advance malting.   The problem resolves itself largely into what type of malt is wanted, and then, with a knowledge of the effects of different malting environments, a method

for obtaining the desired type may be outlined.   Greater recovery may be had by using low temperature and low moisture for a shorter period of malting time, but this is not likely to give good modification as measured by the amount of wort nitrogen.   More wort nitrogen may be produced with almost the same procedure that produces higher diastase, namely, high moisture and low temperature for 6 days or longer.   If the production of a moderately good malt in five days is desired, the following conditions could be considered: malting temperature of about 16°C. (steep temperature of 12°–16°C.) and intermediate moisture of about 46%.   If less time for malting is desired, a moisture increase could be suggested or a small increase in malting temperature probably would compensate in part for reduced time.   If time is not a factor in plant operation, low moisture (43%–44%) and low malting temperature (12°C.) would give high extract, diastase, and wort nitrogen for 8 days' growth and at the same time give a high recovery.

Earlier in this paper reference was made to the possibility of changing malting conditions systematically or periodically.   It seems likely that malting methods prescribed after such a series of experiments would more likely approach maximum quality of malt and full plant efficiency than constant conditions as studied in these investigations. Nevertheless to approach the question of malting methods from the standpoint of constant conditions provides a basis both for interpretation of environmental influences and for planning additional experiments for study of certain fluctuating conditions.

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A STUDY OF SOME DIFFERENCES IN GLUTEN PROPERTIES EXISTING BETWEEN VARIETIES AND TYPES OF WHEAT

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Quality evaluation of wheat varieties is a difficult problem for the cereal chemist. Among the various factors which are included in the term "quality" the biochemical properties of the gluten rank high in importance. It is the combined effect of these properties that are registered in the behavior of the dough during fermentation, as well as in the quality of the finished loaf. While the properties of the baked loaf produced through the use of a suitable baking formula are still considered to be a final measure of flour quality, the employment of various forms of dough-testing devices that attempt to evaluate some physical property or properties of the dough or gluten is becoming more general. Dough-mixing studies appear to be among the more promising of these physical methods, and marked differences in the characteristics of mixer curves obtained from different varieties of bread wheats have been found. These curves, however, do not yield pertinent information in regard to the more fundamental properties of the wheat gluten protein, but tend to measure the effects of many variables.

The viscosity of flour-water suspensions in relation to wheat quality has been investigated by a number of workers. Gortner and Doherty (1918), Sharp and Gortner (1923), Lüers and Ostwald (1919, 1920a, 1920b), Hendel and Bailey (1924) and other workers have studied this phase of the problem and reference is made to their published works for further information in connection with viscosity studies in relation to baking strength.

Gortner, Hoffman, and Sinclair (1929), Gortner (1927), Geddes and Goulden (1930), Harris (1931), and Mangels (1934) have studied the relationships involved in wheat-protein peptization and wheat-quality evaluation. The final conclusion to be derived from these studies is that crude protein content is as indicative of baking strength as any information likely to be obtained from protein-peptization studies.

Changes in rotatory power of protein solutions prepared from wheats of different quality by the method of Osborne were examined by Woodman (1922) and Blish and Pinckney (1924). The results obtained by these workers were in agreement as to the identity of the

gliadins isolated from different wheats but did not agree respecting the glutenin fraction. Kent-Jones (1926) investigated glutenin by this racemization method. Little difference was found in the specific rotations of glutenins prepared from English and Manitoba wheats except in the case of wheats of extreme divergence in baking strength. This worker postulated that any chemical differences which do exist do not suffice to explain variations in baking strength.

Blish and Sandstedt (1933) separated what appeared to be three distinct proteins from wheat gluten. Harris and Bailey (1937) repeated their procedure on a number of glutens prepared from flours of marked differences in baking strength. Statistical analysis of the resultant data showed a significant correlation between gliadin content and loaf volume.

The method of dispersing wheat gluten in sodium salicylate solution rather than the usual solvents, acid and alkali, was advocated by Rose and Cook (1935). Harris (1937, 1938, 1938a) and Harris and Johnson (1939, 1940) employed gluten dispersions in measuring the effects of proteolytic enzymes and flour oxidizers upon wheat gluten properties. Various types and classes of wheat were also examined with respect to possible differences in the biochemical properties of the gluten dispersions, and such attributes as protein dispersibility, quantity of protein thrown down from the dispersions by $MgSO_4$, viscosity, etc were determined. Marked differences in these properties were obtained by varying the enzyme treatment. Significant changes were found in these properties among wheat varieties.

The present investigation was undertaken with the purpose of confirming the results obtained from previous work by studying a larger number and range of samples from another crop year. A rotary shaker had been constructed which rendered it possible to obtain more uniform shaking of the flasks while dispersion was proceeding, than had been achieved in the instance of the work already reported. This shaker was described by Harris and Johnson (1940a), and used by them in work of this nature. It was also deemed advisable to determine the "resistance values" of the glutens prepared from these wheats and to ascertain whether significant differences in this property existed between the various wheats included in this study.

Experimental Material and Methods

Fifteen samples of wheat were collected from different wheat-growing regions of the continental United States. These samples comprised representatives of varieties of the hard red spring, durum, hard red winter, soft red winter, and white wheat classes. The states represented were North Dakota, Kansas, Nebraska, Indiana, and

Washington. The wheats were cleaned, graded, and milled into straight-grade flour on an Allis-Chalmers experimental mill. The humidity of the mill room was controlled at approximately 60% during the milling process. The resultant flours were baked by the malt-phosphate-bromate method with 5% of sucrose, instead of 2.5% as originally required by the standard basic formula. Protein, ash, and moisture determinations were run on the flours.

These flours were also mixed into doughs, using the standard basic formula with 5% sucrose. The doughs were prepared with the Hobart-Swanson mixer, three-minute mixing times being used. Immediately following mixing, the gluten was washed from the dough under a small standardized stream of 0.1% sodium phosphate solution of pH 6.8 by the method of Dill and Alsberg (1924). The glutens were allowed to stand for 15 to 30 minutes under the phosphate solution, and were then divided into the required quantities and dispersed in 10% sodium salicylate solution by constant agitation in the electric rotary shaker for several days. The speed of this machine is adjusted so as to obviate any possibility of foaming the dispersions, with consequent danger of surface denaturation of the protein. A number of gluten concentrations were used to obtain data suitable for representing the relationship between viscosity and concentration. When the dispersion process appeared to be complete the dispersions were centrifuged to remove undispersed protein and starch material and the protein content ascertained by the Kjeldahl-Gunning procedure. Viscosity was determined by means of an Ostwald capillary pipette at a temperature of 25°C. The quantity of gluten protein thrown down by the addition of 6% by volume of concentrated $MgSO_4$ solution to the dispersions also was found, by a method described by the authors in previous publications.

"Resistance" tests were run on a 15-g. portion of the crude gluten by means of the "tenderness tester" described by Binnington, Johannson, and Geddes (1939) and used by Harris and Johnson (1940a) in evaluating differences in resistance between glutens treated with varying quantities of proteolytic enzymes. Reference is made to these papers for the methods involved in obtaining the resistance values.

A further series of determinations was made to find the relative rates at which the glutens prepared from these wheats as described would disperse in sodium salicylate under constant shaking. The rate of dispersion was measured by increase in viscosity as found by the Ostwald pipette.

Discussion of Results

In Table I are presented the origin and description of the wheats used in this investigation, with comparative milling and baking data

obtained from them.   The wheats are grouped according to class and are arranged within the group by increasing crude protein content. These wheats were grown during the 1939 crop season and were sound and free from damage of any nature.   Explanations of loaf appearance are included in the table to facilitate an understanding of the results obtained.

TABLE I

COMPARATIVE MILLING AND BAKING DATA ON A NUMBER OF WHEAT VARIETIES INCLUDED IN THIS STUDY

Data arranged according to increasing wheat protein.   All quantitative data on 13.5% moisture basis.

Lab. No.	Origin	Variety	Test weight	Flour yield	Absorption	Loaf volume	Texture[1]	Crumb color[2]	General appearance	
									Crust[3]	Symmetry[4]
			lbs./bu.	%	%	cc.				
			HARD RED SPRING							
39–190	Mandan, N. D.	Vesta	61.2	73.5	57.8	490	6.0 C, o	6.8 y	S	3.0 o
–158	Langdon, N. D.	Premier	58.2	70.3	58.1	685	7.5	7.5	SlD	4.5 o
–149	Langdon, N. D.	Thatcher	55.5	69.9	59.6	880	6.5 o	7.0 y	SlD	4.5 o
–257	Hettinger, N. D.	Thatcher	57.7	68.2	59.7	940	6.0 o	7.0 y	S	4.5 o
			HARD RED WINTER							
39–274	Kansas	Tenmarq	61.5	71.7	54.0	595	7.5	8.2	S	4.0 o
–276	Kansas	Chiefkan	61.8	73.0	58.4	655	6.5 o	7.5 g, y	S	4.2 o
–281	Nebraska	Cheyenne	63.2	72.1	55.1	655	7.2	7.8	S	4.0 o
–275	Kansas	Blackhull	61.0	68.5	55.7	715	7.0 o	7.8	S	4.5 o
–278	Nebraska	Nebred	59.8	68.3	53.8	780	7.0 o	7.2	S	4.5 o
–277	Kansas	Turkey	61.5	68.5	55.3	720	6.5 o	7.0 y	S	4.5 o
			WHITE WHEAT							
39–116	Washington	Federation	60.8	66.0	52.2	440	3.5 C, o	5.0 g, y	P	2.0 o
–115	Washington	Early Baart	63.5	66.0	55.6	510	6.5 o	7.0 y	P	2.5 o
			SOFT RED WINTER							
39–99	Indiana	Wabash	64.2	68.7	51.3	485	6.5 o	7.0 y	P	2.5 o
			DURUM							
39–279	Mandan, N. D.	Mindum	60.2	69.9	54.6	490	4.5 C, o	4.5 y	Dull	2.5 o
			EMMER							
39–280	Mandan, N. D.	Vernal	31.8	47.2	49.8	510	3.0 C, o	3.5 g	Dull	2.0

[1] Texture: o, open; C, coarse; c, close.   Perfect score, 10.
[2] Color: y, yellow; gy, grey-yellow; g, grey.   Perfect score, 10.
[3] Crust: D, dark; S, satisfactory; SlD, slightly dark; P, pale.
[4] Symmetry: o, overoxidized.   Perfect score, 5.

From the results obtained with the hard red spring wheats, it is evident that a great disparity exists between the highest and lowest loaf volumes. The two Thatcher samples were outstandingly good, while the Premier sample was average in baking strength. The sample of-Vesta, however, was of poor, unsatisfactory strength and yielded a loaf of low volume and inferior symmetry as compared to the other hard wheats. It should be pointed out that a number of the wheats grown at Mandan in 1939 did not measure up to the usual standard of North Dakota spring wheats as a result of climatic effects, and the inferior showing of this wheat should be attributed to unsatisfactory environmental conditions rather than entirely to heritable deficiencies in the variety. The protein content as shown in Table II was also low. The hard winter wheats did not show as great variability in loaf volume as the spring wheats. Nebred produced the largest loaf, while Chiefkan was decidedly inferior in crumb color. The test weights for this group of wheats were very satisfactory. The absorption was slightly lower than the average for the spring wheats. Chiefkan had the highest flour yield and water absorption of the group. These wheats were unfortunately not grown under comparable conditions and no doubt large variations in quality and properties were introduced by environmental differences. The relatively poor showing of Tenmarq could probably be attributed to lower protein content caused by such environmental factors. It produced, however, a loaf with the best crumb color in the entire series of wheats, and its texture rating was also relatively high. The white and soft wheats did not show up well in the baking test, owing principally to their low protein content. The durum and emmer also proved unsatisfactory in quality of bread produced, as might be expected from these types of wheat, which have been found unsuitable for the production of modern bread. The emmer had a very low test weight and flour yield, caused by the proportionately large outer husk of the kernel. This circumstance also led to the flour having a higher protein content than the wheat, as shown in Table II.

.In Table II are shown the crude wheat and flour protein, flour ash, resistance values, the ratio of loaf volume to flour protein, and the specific volume (ϕ/C) as calculated from the viscosity data by the equation of Kunitz:

$$\eta/\eta_0 = \frac{1 + 0.5\ \phi}{(1 - \phi)^4}$$

where η is the coefficient of viscosity of the protein sol, η_0 the coefficient of viscosity of the dispersion medium, ϕ the percentage of the system occupied by the volume of the disperse phase, and C concentration of the disperse phase. These specific volumes, or the

volume occupied by 1 g. of protein, are the average values computed for each wheat variety, and there are accordingly a number of observations included in each. The use of the average value seemed to be justified inasmuch as no great variability was evident among the

TABLE II

WHEAT AND FLOUR PROTEIN, ASH, RESISTANCE VALUES, AND SPECIFIC VOLUMES

Data arranged in order of increasing wheat protein within class.

Lab. No.	Variety	Origin .	Crude protein (N ×5.7)		Ash	Resistance value	Loaf vol./protein content	Specific volume
			Wheat	Flour				
			%	%	%	sec.		φ/C
HARD RED SPRING								
39–190	Vesta	Mandan, N. D.	11.6	11.3	0.48	81.4	43.40	7.97
–158	Premier	Langdon, N. D.	15.3	15.0	0.54	73.0	45.66	7.91
–149	Thatcher	Langdon, N. D.	16.4	16.0	0.52	82.4	55.00	7.93
–257	Thatcher	Hettinger, N. D.	17.5	17.3	0.51	86.0	54.34	7.46
HARD RED WINTER								
39–274	Tenmarq	Kansas	14.1	13.2	0.38	70.0	45.08	7.62
–276	Chiefkan	Kansas	15.8	15.3	0.43	56.8	42.81	7.11
–281	Cheyenne	Nebraska	16.2	14.6	0.47	81.0	44.86	7.44
–275	Blackhull	Kansas	16.2	15.8	0.45	49.4	45.25	7.53
–278	Nebred	Nebraska	16.9	16.2	0.42	71.0	48.15	7.80
–277	Turkey	Kansas	17.2	16.9	0.58	45.8	42.60	7.10
WHITE WHEAT								
39–116	Federation	Washington	10.8	9.3	0.40	106.0	47.31	7.60
–115	Early Baart	Washington	11.1	9.7	0.44	75.6	52.58	7.96
SOFT RED WINTER								
39–99	Wabash	Indiana	10.1	8.6	0.48	61.0	56.40	6.94
DURUM								
39–279	Mindum	Mandan, N. D.	15.3	14.6	0.56	53.8	33.56	5.82
EMMER								
39–280	Vernal	Mandan, N. D.	12.9	16.8	0.68	29.0	30.36	6.28

specific volumes for each wheat. No consistent trends toward a change in hydration with increasing protein concentration were noticeable. The ratio of loaf volume to flour protein was calculated to obtain a value more representative of the baking quality of the

wheats, inasmuch as some of the samples possessed a high protein content but would yield poor loaves because of inferior gluten quality, while other samples would be penalized as a result of low protein content although the gluten quality might be very high.

A substantial range in wheat protein content is evident, varying from 17.5% for one of the Thatcher samples to 10.1% for Wabash, a difference of 7.4%. The corresponding flour protein range was from 17.3% to 8.6%, a difference of 8.7%. The range in ash content was from 0.68% to 0.38%, a difference of 0.30%. The emmer was extremely difficult to mill and this fact, no doubt, tended to increase the ash for this particular sample. Tenmarq produced the lowest flour ash of any of the wheats. The resistance values, as determined on the "tenderness tester," represent the time in seconds for the plunger of the instrument to compress the gluten sample to a thickness of 0.175 inch and was calculated from a curve traced by a kymograph. This method of calculation has been explained by Harris and Johnson (1940a). The stiffer the gluten, the longer this time will be, while a relatively short time in seconds denotes a soft nonresistant gluten. Federation, a soft white variety grown in the state of Washington, had the most resistant gluten, being substantially higher in this respect than the other samples. The sample of emmer, on the other hand, had a soft gluten and the plunger was able to compress this gluten quite readily. The hard red winter wheat varieties showed a surprising variability in this gluten property, ranging from 81.0 seconds for Cheyenne to 45.8 seconds for Turkey. No relationship between this value and the commonly accepted quality ratings of the hard wheats is evident from the data. It appears, however, that the hard red spring wheats as a class have more resistant glutens than the hard red winter wheats.

The ratio of loaf volume to flour protein does not show the differences that are evident in the loaf volumes. This would be expected, since the variability in flour protein will in many cases largely compensate for the loaf-volume differences. The durum and emmer values are, however, distinctly lower than the ratios for the other samples.

The specific volumes, shown in the right hand column of Table II, vary appreciably among the different classes. The hard red spring wheats appear to be slightly higher in specific volume than the hard red winters. The white wheats also are high in this gluten property. The last three wheats in the table, the soft winter, durum, and emmer, are distinctly lower in specific volume. It is evident from these data that this property of the white wheat glutens is quite different from

the specific volume of the soft red winter wheat gluten micelles and resembles the hard wheats.

The data obtained from the viscosity determinations are presented in the form of curves rather than in the form of tables, for greater ease of interpretation. Figure 1 shows the relationship between viscosity and protein concentration for representative samples of different classes. Average values were calculated for each of the hard wheat
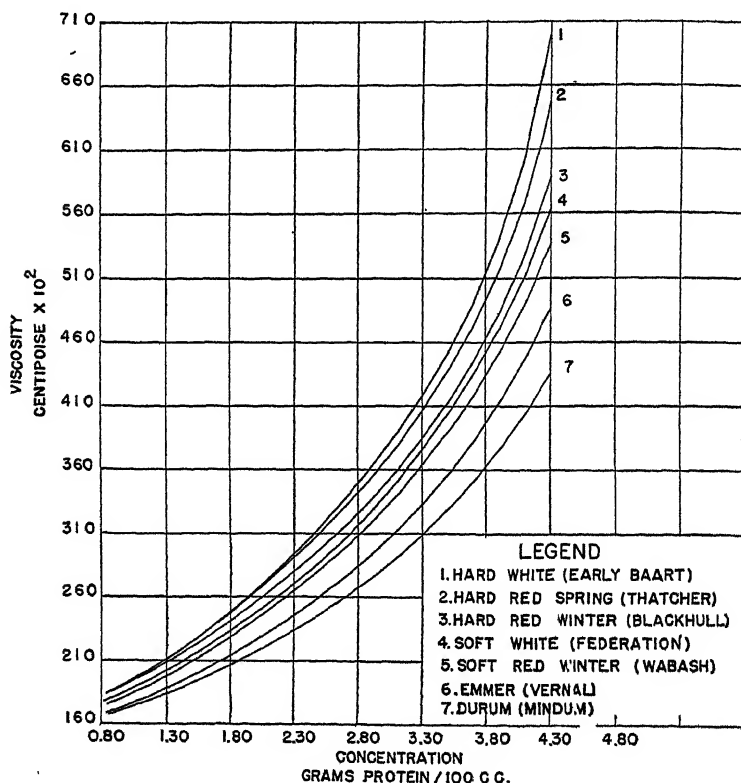
Fig. 1. Relationship between viscosity and gluten protein concentration in different wheat classes.

classes and the variety having the viscosity curve most similar to the average of the class was chosen to represent the class, since it would be clearly impossible to present in one figure the data from all the wheats studied without hopelessly confusing the picture. The curves show that Early Baart, a hard white wheat, has the highest viscosity of the wheats investigated. The hard red spring wheat has the second-highest viscosity, followed at an interval by the hard red winter wheat. Federation, a soft white wheat, has a curve intermediate in

position between the curves obtained from the hard red winter wheat and Wabash, a soft red winter wheat. Emmer has the second lowest and durum the lowest viscosity of the various classes investigated. The viscosity curves of the sodium salicylate gluten dispersions of these two latter wheats appeared to show marked divergence from the
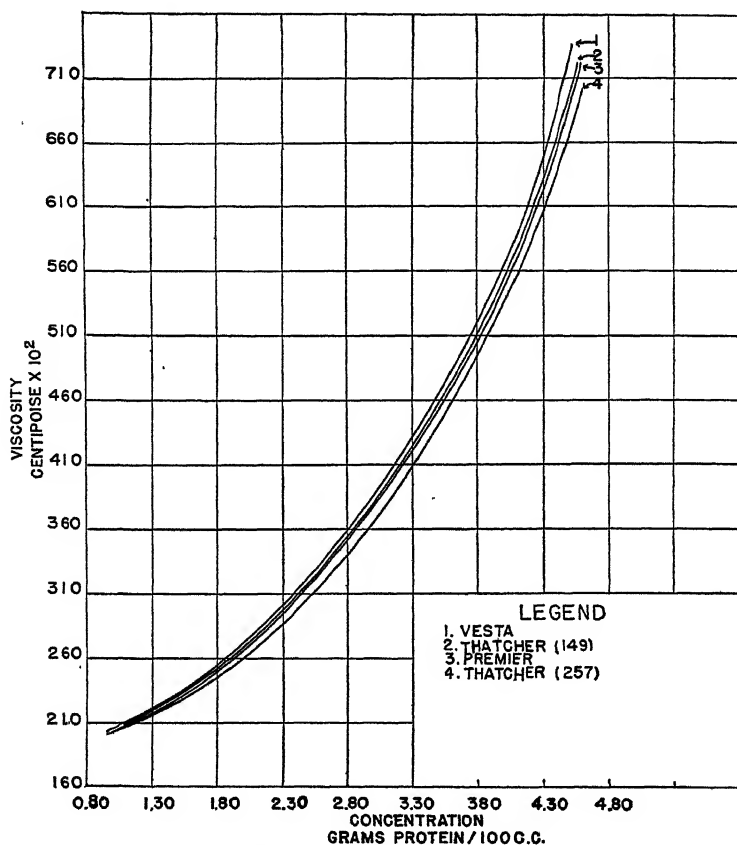
Fig. 2. Relationship between viscosity and gluten protein concentration for hard red spring wheats.

curves of the other wheats presented in the figure, and are no doubt connected with fundamental genetic differences. In many instances it will be found that hard red winter wheats give gluten dispersions of viscosities equal to those of some of the hard red spring wheats.

In the instances of soft red winter, emmer, and durum wheats these differences in the viscosity curves are due, no doubt, to a progressive decrease in size of the gluten micelle as shown in the specific

volume data presented in Table II. It would appear that the white wheats tend to have larger protein micelles in their gluten dispersions than would be expected from their relative baking strengths.

Figure 2 represents the viscosity-concentration curves obtained from the sodium salicylate dispersions of the hard red spring wheat
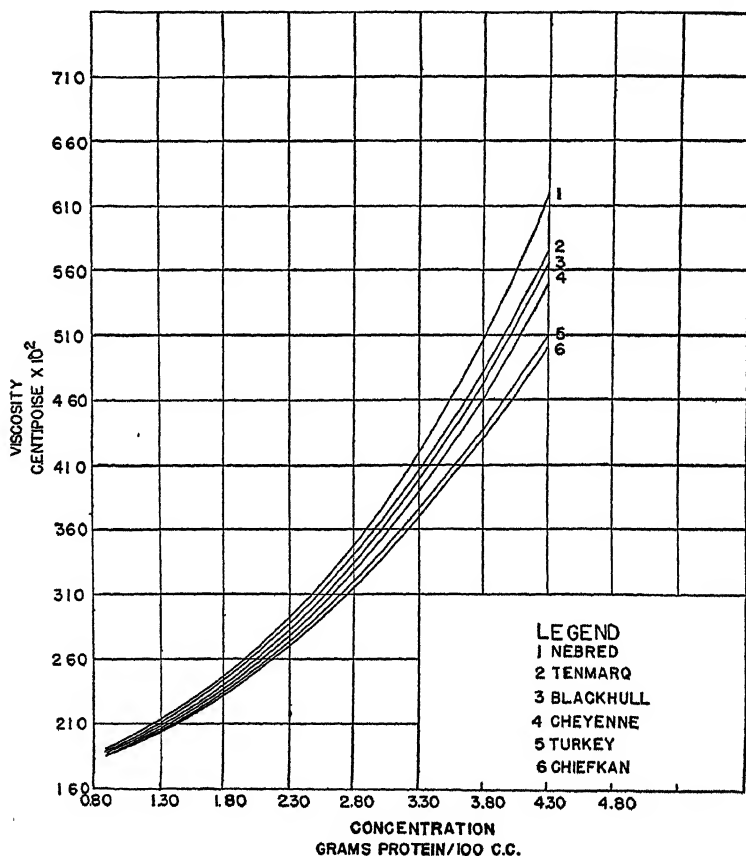
Fig. 3.   Relationship between viscosity and gluten protein concentration for hard red winter wheats.

glutens. Only minor differences are shown in spite of the great disparity in loaf volume between Vesta and Thatcher. It would be expected, from a knowledge of its baking behavior and the "feel" of the gluten during the washing process, that Vesta had the gluten present in a more or less coagulated condition and that accordingly a high viscosity curve would result. Such a picture, however, is not found in Figure 2 in view of the small differences shown in the viscosity-

concentration relationships. It is therefore evident that, while class differences may be defined by these curves derived from viscosity studies on wheat-gluten dispersions, differences in baking strength within the class would not be shown. No significant differences in specific volumes of the micelles in dispersions prepared from various hard red spring wheats were to be noted.
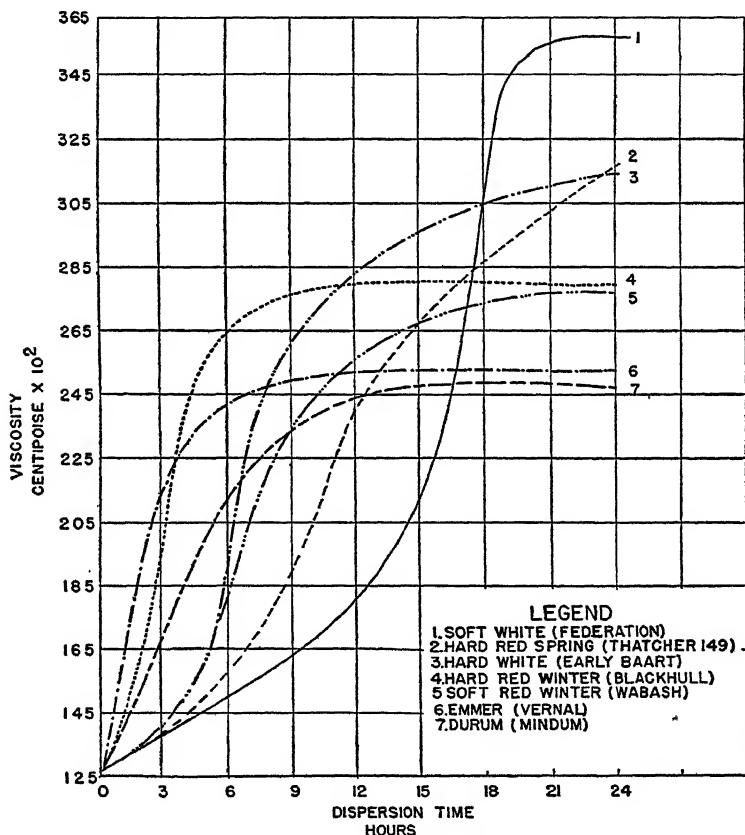
Fig. 4. Increase of viscosity with time of dispersion—glutens prepared from wheats of different classes.

Figure 3 shows the viscosity-concentration curves for the hard red winter wheat varieties. Nebred has the highest curve and is very close to the average value for the hard red spring wheats. Tenmarq is next in order of viscosity, while Blackhull and Cheyenne are similar. It was rather surprising to find Turkey distinctly below the first four varieties and quite close to Chiefkan in viscosity values. In similar

investigations with 1938 crop samples, Harris and Johnson (1940) found that a sample of Kansas Turkey ranked very close to the hard red spring wheats in respect to the viscosity-concentration curves, and was substantially higher than a Chiefkan sample. In the present instance the lower position of the curves of these two varieties is due to significantly smaller gluten protein micelles as compared with the other hard winter wheat varieties.
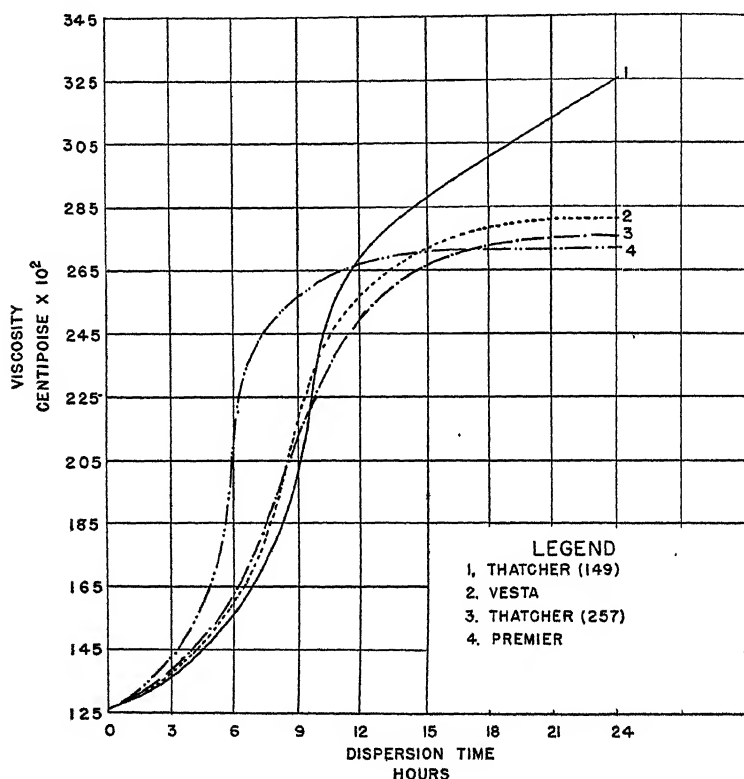
Fig. 5. Increase of viscosity with time of dispersion—glutens prepared from hard red spring wheats.

Figure 4 shows the relative dispersion rates obtained on glutens prepared from wheats representative of the different types included in the study. These glutens were washed from doughs made up from the standard basic formula with 5% of sucrose and the glutens were washed immediately after the completion of mixing of the doughs. It is at once apparent from the figure that large differences exist among the wheats in respect to gluten-dispersion rate. Federation gluten was very resistant, at first, to dispersion, but it disintegrated at a

rapid rate after approximately 15 hours in the shaker, and reached the highest viscosity of this series of dispersions.   The hard red spring wheat gluten was next in order of resistance but the shape of the curve was quite different from that of Federation and shows a more consistent rate of dispersion.   The soft red winter wheat gluten was next in order of dispersibility, while Early Baart, although similar at first, showed more resistance after the sixth hour.   Emmer and Blackhull
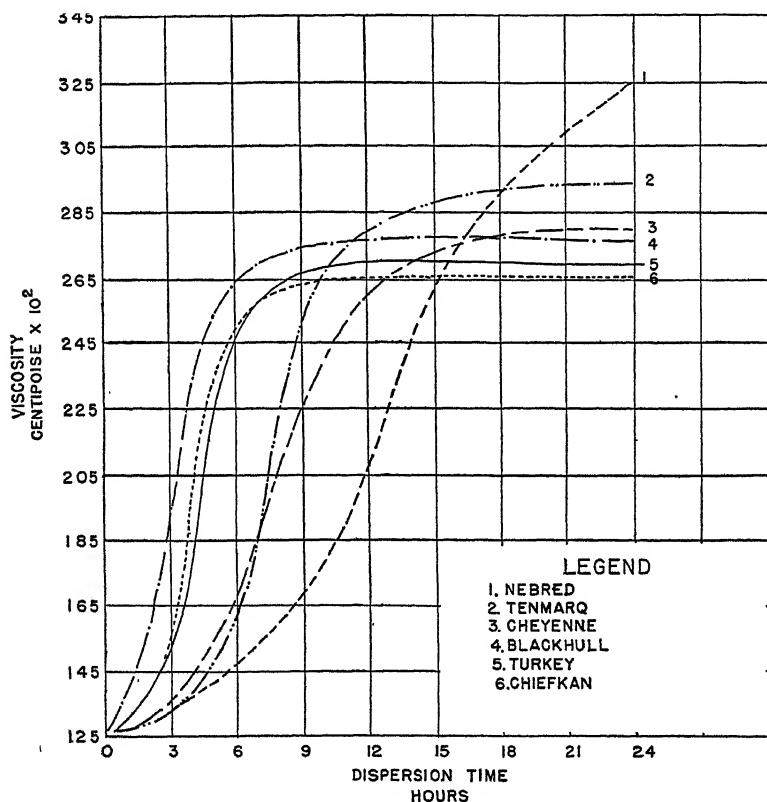
Fig. 6.   Increase of viscosity with time of dispersion—glutens prepared from hard red winter wheats.

dispersed rapidly, while the durum gluten dispersed more slowly than either.   It is evident that Federation has a gluten definitely different from the other wheats represented, in respect to dispersibility in 10% sodium salicylate.   It is probable that the state of protein aggregation, or particle size, is related to dispersibility, the larger particles being more resistant.

Figure 5 represents the dispersion rates of the four hard red spring wheat glutens prepared as in the previous instance.   The curves are,

to some extent, similar in their general characteristics, especially if the variety Premier is excepted. This is a new variety that has not been distributed in North Dakota and has generally been found to be of lower baking strength than the other two varieties represented. It shows a tendency to disperse more readily than the other hard red spring samples. The sample of Vesta, in spite of its unsatisfactory showing in the baking test, was very similar to the Thatcher sample in respect to dispersibility.

In Figure 6 the results obtained with the hard red winter wheat varieties are presented. Nebred, a comparatively unknown variety, is the most resistant to dispersive action and resembles the spring wheats in this particular. Tenmarq and Cheyenne are somewhat similar, but the former appears to disperse at a lower rate subsequent to the seventh hour. The remaining three varieties apparently fall into a group of very similar dispersibility, but Blackhull has the least resistance. This behavior is probably linked with the low mixing tolerance of this variety.

TABLE III

TABLE OF STATISTICAL CONSTANTS—MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY

Variables	Means	Standard deviations	Coefficients of variability
Flour protein, %	14.04	2.832	20.17
Loaf volume, cc.	636.67	147.509	23.17
Viscosity, centipoises $\times 10^2$	319.02	94.778	29.09
Fractionation value	1410.20	669.862	47.50
Resistance value, seconds	68.15	18.538	27.20
Specific volume, ϕ/C	7.36	0.609	8.27

In Table III are shown the means, standard deviations, and coefficients of variability computed from the data. With the exception of the specific volume, the results indicate a high degree of variability among the wheats in respect to the values determined. The quantity of protein removed from the dispersions by 6% (by volume) of concentrated $MgSO_4$ solution was more variable than any other value examined.

Table IV presents the correlation coefficients which were calculated between the more pertinent variables considered in this investigation. The relationship between viscosity and fractionation value is extremely high and justifies the prediction of the latter variable from a knowledge of the former. Similar conclusions were reached by the authors in a previous study (Harris and Johnson, 1940a), who found values of $+ .8392$ ($N = 30$) and $+ .9592$ ($N = 35$) for this relationship. There

are also significant positive correlations between flour protein and loaf volume, resistance value and specific volume, resistance value and loaf volume/flour protein, as well as between the latter ratio and specific volume. In other words, the larger the specific volume, the higher the ratio and the greater the resistance of the gluten to compression. Because of extreme divergence in baking quality due to the inclusion of wheats of inferior baking strength in the study, it was not thought advisable to determine the correlation between these variables and flour protein or loaf volume, but rather to use the ratio of loaf volume to flour protein. The results of this study lead to the concept that larger micelles are present in the glutens of wheats of superior baking quality, but the reservation must be made that this relationship does not appear to be valid for varieties within the better quality bracket.

TABLE IV

CORRELATION COEFFICIENTS COMPUTED FROM THE DATA

Significant correlation coefficients are in bold type.

Variables correlated		Correlation coefficient r_{xy}	Probability P
X	Y		
Flour protein, %	Loaf volume, cc.	+.7251	<.0001
Viscosity, centipoises $\times 10^2$	Fractionation value	+.9706	<.0001
Flour protein, %	Resistance value, seconds	+.0424	>.5542
Resistance value, seconds	Loaf volume, cc.	+.0966	>.5542
Resistance value, seconds	Specific volume, ϕ/C	+.6518	.0084
Resistance value, seconds	Loaf volume/flour protein	+.6007	.0179
Loaf volume/flour protein	Specific volume, ϕ/C	+.6604	.0074

A direct relationship between the specific volume of the gluten micelle in 10% sodium salicylate and dispersibility does not seem to be indicated by the results. The durum and vernal emmer, however, which have the smallest specific volumes, also tend to possess a high dispersion rate. It must be borne in mind with respect to the results obtained upon protein dispersions in sodium salicylate that the environment surrounding the protein micelle is different from that which exists in the native condition. There is reason to suspect that chemical combination may exist between the protein particle and sodium salicylate molecule, which may alter, to some extent, the size and configuration of the particle but should not change the relative size of the particle from different wheat glutens. At least it has been shown by Rose and Cook (1935) and Cook and Rose (1935, 1935a) that solutions of sodium salicylate caused little denaturative changes in gluten as compared to the solvents, acid and alkali.

The resistance of the washed gluten, as determined by a "tenderness tester," was not related to loaf volume but was correlated with the ratio of loaf volume to flour protein and with specific volume in this investigation. It is doubtful whether this test has any utility for purposes of determining baking strength, inasmuch as the baking test would differentiate wheats of different strengths in a more precise and readily understandable manner. It may have a certain limited value as a supplementary test to determine the effects of proteolytic action or dough fermentation upon certain physical properties of the gluten.

The measurement of rate of gluten dispersion in 10% sodium salicylate solution during constant, gentle agitation, appears to yield as informative results in relation to fundamental gluten quality as any test described in this paper. It appears to offer possibilities as a means of supplementing the baking test with additional and intimate information regarding gluten quality. Further investigations are in progress to clarify these relationships and to delimit more clearly the applicability of the test.

Summary and Conclusions

Glutens were washed from doughs prepared from 15 flours experimentally milled from wheats comprising varieties of the hard red spring, hard red winter, soft red winter, durum, and white wheat classes, and emmer. The flours were also baked by the malt-phosphate-bromate method. A large range in baking strength was covered by the 15 wheats included.

Viscosity studies were conducted upon the glutens dispersed in 10% sodium salicylate solution using an Ostwald capillary pipette at 25°C. The protein concentrations of these dispersions were determined and viscosity-concentration curves constructed. The hydrodynamic and specific volumes of the protein micelles were calculated by an application of the equation of Kunitz. The hard red spring wheats had higher viscosity on the average than the hard red winter wheats, while the soft red winter was the lowest of the three. Emmer was next lowest to the soft red winter in respect to viscosity, while durum was the lowest of the wheats studied. Early Baart, a hard white wheat, had the highest viscosity of any variety in the study.

The relative placing of the soft red winter, emmer, and durum wheats in relation to the viscosities of their dispersions at equal protein concentrations appeared to be due to a progressive decrease in micelle size with consequent decrease in viscosity, as shown by the values computed for their specific volumes. No significant differences are shown among the four red spring wheat varieties studied, but in the

hard red winter wheat class, Turkey and Chiefkan fell below the other four samples in the viscosity and specific volume of the gluten particles in 10% sodium salicylate dispersion. Gluten dispersion rates in sodium salicylate were obtained and graphs constructed to represent the differences between classes and varieties. Large differences are evident between wheat classes as well as between some of the hard red winter wheat varieties. The hard red spring wheats did not show any very marked differences, however. Nebred, a comparatively new variety, was the most resistant to dispersive action, while Blackhull had apparently the least resistance. Turkey and Chiefkan were similar. No consistent changes of specific volume with increasing protein concentration were shown by the data.

While the hydrodynamic or specific volume of the protein particles of these wheats in sodium salicylate colloidal solution does not appear to be definitely connected with their dispersibility in sodium salicylate, it is nevertheless evident that the soft red winter Wabash, emmer, and durum have significantly lower specific volumes in their dispersions and would have the highest dispersion rates. It would also appear that the varieties of wheat that are resistant, or have a high degree of mixing tolerance, will have a relatively low dispersion rate. Wheats that lack mixing tolerance will tend to be easily dispersed in 10% sodium salicylate. A measurement of gluten resistance to compressibility as determined upon the "tenderness tester" did not appear to yield pertinent information in regard to quality differences. The baking test appeared to be more sensitive to variety differences and also appeared to yield data more readily interpreted into quality concepts.

The determination of fractionation value, or the quantity of protein removed from the dispersions by the addition of a definite concentration of $MgSO_4$, was highly related to the viscosity of the corresponding colloidal solutions. In view of the labor and reagents involved in the measurement of fractionation value as contrasted with the determination of viscosity, it would seem that viscosity determinations alone should be satisfactory to use in studies of this nature.

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BOOK REVIEW

What Are the Vitamins? By Walter H. Eddy. Published by Reinhold Publishing Company, New York, N. Y. 230 pages. 1941. Price $2.50.

This book, in the words of the author, is an expression of his personal views of what seems pertinent and reliable information from past and current investigations in the vitamin field. The author has assembled, condensed, and presented a great deal of information selected from the enormous volume of literature on vitamins. The book naturally assumes some knowledge of chemistry and biology on the reader's part, as it would be quite impossible to discuss, for instance, the pathological aspects of the vitamin deficiencies in ordinary lay language.

The book encompasses thirteen chapters, giving first the historical background of development of the vitamin field, accompanied by a brief review of all the vitamins, including those isolated and those postulated. This section is followed by a discussion of the mode of action of various vitamins, including the biological processes or reactions they enter into. A number of structural formulas are given here but it is regrettable that many errors appear in their presentation. These errors detract seriously from the value of the book as a handy reference. In some respects the text contains gaps in continuity which make it somewhat difficult to follow.

The next section discusses the physiological properties of the vitamins and the effects caused by a deficiency of a particular vitamin in the diet. It also lists the consensus of expert opinion regarding daily human requirements for each vitamin.

The chapters following are devoted to the functions and properties of each of the several vitamins. Many references to published scientific literature are used in the text with a corresponding bibliography at the end of each chapter. This part of the book should prove very useful to those wishing to make a survey of the vitamin literature.

Appendix A is more or less a recapitulation of the chemical nature and structural formulas of the vitamins presented earlier in the text. Again, a few inaccuracies in structural formulas occur but not as seriously as those in Chapter II. Appendix B is a table of vitamin values of common foodstuffs along with definitions of vitamin units and equivalents in weight of the pure chemical compound wherever this is possible.

Good author and subject indexes are provided.

<div align="right">G. Ivor Jones</div>

BOOKS ON SOILLESS GROWTH OF PLANTS

Aside from the present popular interest in growing plants without soil, the possibilities that the several techniques offer for studying the relationships between plant nutrition and the characteristics of cereal grains merit attention. A number of workers have already demonstrated that soil fertilization markedly influences the quantities of protein and phosphorus in wheat and wheat flour. The effect of fertilizer treatments on the "quality" of the wheat and flour constituents is still problematical, but it is not unreasonable to assume that here too there must be a relationship.

The small-scale sand and water culture equipment used in the past has limited the amount of wheat produced under absolutely controlled nutrition to quantities

insufficient for adequate milling and baking. During the past few years techniques have been developed that facilitate production on such a scale that large samples of grain may be made available. A combination of present micro methods of milling and baking with large-scale nutrient culture now seems to be practical. Such a technique offers an approach which may well solve many of the problems associated with determining the relationship between plant nutrition and baking quality of the resulting grain. It is with this thought in mind that reviews of the following five books are presented for the attention of cereal chemists.

Chemical Gardening for the Amateur. By Charles H. Connors and Victor A. Tiedjens. Published by Wm. H. Wise & Co., New York, N. Y. 1939. 255 pages. Price $1.95.

This book should prove very valuable to the amateur "chemical gardener." It is written particularly for those interested in the hobby of growing plants without soil. However, the excellent illustrations, good binding, and wealth of material applicable to amateur gardening of all kinds make it a welcome addition to any book shelf. Too, an attempt is made to correct some of the prevalent misconceptions regarding soilless growth of plants.

The authors' authoritative discussion is supported by a high degree of accuracy in their factual material. In addition the presentation is good and the book is entirely readable. Of the nine chapters, five deal specifically with chemical gardening and, of these, three give detailed information and methods. The remaining chapters are more general in nature and in them the authors present a little philosophy, some condensed plant physiology, and a lot of practical information for amateur gardeners. The authors' viewpoint is that chemical gardening is valuable chiefly as a hobby and their discussion is, therefore, of particular value for those interested in that aspect. There is some deficiency in detail as regards the setting up of any particular method, which deficiency would handicap a worker interested more in mass production than in experimentation with methods. Appendices containing planting information, weights and measures, glossary, and index make the book complete.

Soilless Growth of Plants. By Carleton Ellis and Miller W. Swaney. Published by Rheinhold Publishing Corporation, New York, N. Y. 1938. 155 pages. Price $2.75.

One of the first books to be published dealing entirely with soilless gardening, this book satisfied a definite need at the time. In spite of more recent contributions it still serves its purpose as an excellent introductory text for the beginner. It offers an interestingly written survey of the various phases of nutrient culture but, like several other books on the same subject, suffers as a result of the attempt to crowd too much material into a small volume.

In addition to chapters dealing specifically with water culture, aggregate culture, and nutrient formulas, an introduction is given to such subjects as general plant physiology, plant diseases, and special chemicals such as plant hormones. Particular attention is given to the applications of soilless gardening methods both in the home and in commercial greenhouses. The placing of such suggested applications into practice is facilitated by the excellent illustrations provided.

In addition to the somewhat too great enthusiasm as to the possibilities of soilless growth of plants, the manner of presentation is to some extent objectionable. Entirely too much information is crowded into a small space, resulting in inadequate and often confusing treatment. Because of this, those chapters not dealing directly with nutrient culture detract from, rather than add to, the value of the book.

The Complete Guide to Soilless Gardening. By W. F. Gericke. Published by Prentice-Hall, Inc., New York, N. Y. 1940. 285 pages. Price $2.75.

Credit for realizing the commercial possibilities of water culture of plants and for popularizing the method must be given to Dr. Gericke. This book presents to many readers their first opportunity to find out what the author has been doing and what methods he proposes. As such, it is a valuable contribution. Further value lies in his illustrations of the wide variety of crops which have been grown by water culture and in the suggestion that the method may be an effective aid for studying the relationship of nutrition to baking quality of wheat. However, the book is offered as a "complete guide" to soilless gardening, whereas it may be considered as such only if the reader accepts the author's tenet that water culture, or "hydro-

ponics," is the only true soilless gardening. Little information relative to the more popular aggregate culture (sand, gravel, cinders) is included.

For those interested in production by large-scale water culture the book should be considered as essential equipment. Its twenty-one chapters cover everything from the best type of joints to use in wooden tanks to a discussion of the pH scale. However, many of the details pertinent to hydroponics are obscured by reason of poor organization and inclusion of much extraneous material not essential to successful practice of the method. The book is well illustrated and has an adequate glossary of technical terms.

The reader should be cautioned that many of the views expressed in the comparisons of soil and water culture are those of the author and are not necessarily accepted by other workers. Particularly is this true of comparisons regarding the economics of production and the yield and quality of the product. The economic practicability of large-scale outside water culture is very questionable except under very unique environmental conditions. Attention should be drawn to an error in the discussion of figure 9. The small seedlings with long roots are obviously those produced by low, not high, nitrogen intake.

Gardening without Soil. By A. H. Phillips. Published by Chemical Publishing Co., Inc., New York, N. Y. 1940. 137 pages. Price $2.00.

This is a very well organized and well written book. It is intended primarily for the use of soilless gardeners in the British Isles. However, with the exception of such material as a list of the English firms which can supply the necessary equipment, the content is applicable to American conditions. As much specific information as is found in some of the longer books is concisely and entertainingly presented. The author stays close to his subject and does not make his book a conglomeration of plant physiology, horticulture, and a number of other subjects.

The six chapters progress logically from a short introduction on "How Plants Grow" through water culture, aggregate culture, nutrient solutions, and the practice of soilless culture, to "Soilless Culture on the Farm." This last chapter on the use of nutrient culture in farming seems somewhat out of place, since it deals with the production of sprouted grain for forage. However, this is an important practice and the use of nutrient solutions to increase the feeding value of the product is an endeavor well worth describing. A scientific paper dealing with the production of horticultural crops by water culture is reprinted as an appendix. The appended bibliography is of value but the book suffers from lack of both an index and a glossary of technical terms.

Growing Plants in Nutrient Solutions. By Wayne I. Turner and Victor M. Henry. Published by John Wiley and Sons, Inc., New York, N. Y. 1939. 154 pages. Price $3.00.

This book is written primarily as an aid to the commercial greenhouse grower. The senior author has had much commercial greenhouse experience and it is stated that in 1939 he had over 70,000 square feet of greenhouse space in nutrient culture. The reviewer's opinion is that no commercial greenhouse man should attempt to convert part or all of his bench space to soilless culture without this book as a guide. In addition much of the information is of value to both amateur and technical experimenters in the field. The book is complete in every respect, including appendices listing sources of equipment and materials and providing a bibliography, sample greenhouse record sheets, and a combined index and glossary.

The first four chapters deal with methods of nutrient culture, their advantages in greenhouse production, and detailed instructions for installation of the widely used subirrigated aggregate culture. Of the remaining eight chapters, four are devoted to the preparation and testing of nutrient solutions, two to rudimentary plant physiology, one to the diagnosis of deficiency symptoms, and one to general cultural conditions. Illustrations are adequate and assist in gaining an appreciation of the method. The considerable discussion of the practical, large-scale regulation of plant growth by modification of the potassium-nitrogen ratio in the nutrient is very welcome. Beginners especially will find the "keys" for diagnosing plant-deficiency symptoms instructive. Altogether an outstanding contribution has been made in bringing before greenhouse men generally this description of nutrient culture in practice.

<div align="right">ERIC KNEEN</div>

THE EFFECT OF STORAGE ON THE PROTEIN OF WHEAT, WHITE FLOUR, AND WHOLE WHEAT FLOUR

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It has been observed on several occasions in this laboratory that not as much protein could be extracted from ground seeds after the meal had stood around for a period of time as from the freshly ground seeds. This decrease in solubility suggested that other fundamental changes in the properties of the proteins may also occur as a result of aging. Because of the meager knowledge available on this subject studies were conducted in order to determine the effects of storage under different conditions on the proteins of several seeds of agricultural importance. This paper presents the results of storage for different intervals during a period of two years on the proteins of wheat kernels, white flour, and whole wheat flour.

Large and increasing quantities of grain are being stored. On July 1, 1940, it was estimated that 270 to 300 million bushels of wheat (new crop) will go under seal. Ten million bushels were in storage on farms under seal, 14 million bushels stored for crop insurance reserve, and 3 million for Commodity Credit Corporation and Federal Surplus Commodities, to be used for relief. At the same time, 400 million bushels of old corn were estimated to be under seal on farms and an additional 65 million bushels stored by the Commodity Credit Corporation. It is important to know the nature and extent of changes that may result during storage and of the effect they may have on the value of the proteins for food and technological purposes.

Large losses are known to be sustained every year through spoilage of grains and other agricultural products as a result of improper storage conditions and methods of handling. Less detectable but highly impor-

tant changes may also occur under apparently satisfactory storage conditions.

It has long been known that wheat and flour undergo certain changes as a result of aging. These changes have concerned primarily the baker and the miller. It is well recognized that flour improves in baking quality during storage for a limited period. It is generally believed that the baking properties of flour, expressed in terms of loaf volume, texture, and other criteria used for denoting baking strength, are intimately associated with, if not dependent upon, the quantity and properties of its gluten—that peculiar complex of the proteins gliadin and glutenin. Heretofore, studies have been confined largely to correlating the effects of storage with changes in baking quality, acidity, diastatic value, viscosity, and other properties. Little data is available on the effect of storage on the fundamental properties of the proteins.

The extensive publications on the effect of storage on the baking qualities of flour have been reviewed by Bailey (1925), and more lately by Cathcart and Killen (1939) in their paper on storage changes in flour.

Information on the nature and extent of the changes that occur in proteins on storage is of importance not only to the baking and milling industry but also in connection with the rapidly increasing production and utilization of proteins for technical purposes. The production yields of the proteins depend largely on their solubility and would be, therefore, largely diminished by denaturation.[1] The suitability of the proteins for the manufacture of specific products such as plastics might well be conditioned by the extent and nature of storage changes. Possible changes in nutritional value are also an important consideration.

Experimental

Preparation of material.—The wheat used in these studies was graded No. 2 Red Winter wheat of the 1937 crop produced in Ohio. It was procured for us through the kind offices of the Milling, Baking, and Chemical Laboratory of the Grain Division of the Bureau of Agricultural Economics, United States Department of Agriculture, and was milled during the week of November 15, 1937, in their experimental flour mill. The wheat was cleaned in an experimental milling separator and scourer. The loss in preparation for milling was approximately 3.5%. The wheat was milled in the usual way by tempering at room temperature in two stages to 15%, and the milling was done without generation of heat in any way.

In the preparation of the whole wheat flour, in order to avoid development of heat during grinding, the wheat was coarsely cracked in

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[1] The term *denaturation* as used in this paper refers only to decrease in solubility of the proteins.

an attrition mill, and the product was then reduced to a fine powder by reduction on the smooth rolls of the flouring mill.

The white flour was what is known as a 95% patent flour. In other words, all the flour possible was removed from the wheat, and the last 5%, which was discolored with germ stock and bran, was discarded. On a percentage extraction basis the wheat yielded 70% of flour.

Storage of material.—On November 24, 1937, samples of the white flour and of the whole wheat flour were placed in sealed Mason jars and in closely woven cotton bags. The samples were stored in sealed cupboards in a room with controlled temperature and humidity. The temperature was maintained at about 76° ± 3° or 4°F. and the relative humidity at about 55%. Samples in sealed jars and in closely woven cotton bags were also stored in a refrigerating room maintained at a temperature of 30°F. Each jar contained approximately enough material to provide for the required analyses at the end of each storage period, thus making it unnecessary to open any jar before the end of the stated storage periods. Samples also of the whole kernels were stored in half-gallon glass jars at 76°F. and 30°F.

Analyses and procedure.—Analyses of the white flour and whole wheat flour samples stored in jars were made at the end of 1, 4, 7, 12, and 24 months of storage, and of those stored in bags at the end of 7, 12, and 24 months. The whole seeds, ground to the approximate fineness of the original whole wheat flour, were analyzed after storage periods of 9 and 24 months.

The different analytical determinations made on the samples were considered suitable to reveal the nature and extent of some of the fundamental changes that may occur in the properties of the proteins during varying periods and conditions of storage. All the determinations were made in duplicate or triplicate, and the results given are averages of closely agreeing values.

The first series of analyses were made on the samples at the time they were placed in storage. The results of these analyses serve as a basis of comparison with similar data obtained later on the stored samples whereby the extent of the changes that occurred may be ascertained. Throughout the investigation special care was taken to have all the corresponding analyses made in the same way and under the same conditions in order to have the results of the different sets of analyses on as comparable a basis as possible. For the analyses of the stored wheat kernels the samples were ground to a fine powder in a hand-driven mill, care being taken to have the material reduced to the same degree of fineness for each set of analyses.

Moisture and total nitrogen.—Determinations were made on the freshly prepared material, and on all samples at the end of each storage

period. The moisture was determined according to the official A.O.A.C. method (1940), by heating about 2 g. of the samples at 130°C. for one hour. Nitrogen was determined in triplicate on 1-g. samples by the Kjeldahl method and the average results calculated to a moisture-free basis.

Free ammonia nitrogen.—Duplicate samples of the meals corresponding to 10 g. of the moisture-free material were transferred to two Claissen flasks (1.5 to 2 liters capacity) and to each flask were added 200 ml. of distilled water and 100 ml. of alcohol. About 8-ml. portions of *n*-butyl alcohol were added in order to prevent excessive foaming. The mixtures were then made slightly alkaline by addition of a 10% suspension of $Ca(OH)_2$, and subjected to distillation under reduced pressure (20 to 30 mm.) for 45 minutes at 40°C. to 50°C. The free ammonia was collected and determined in 12 to 20 ml. tenth-normal H_2SO_4 according to the method of Van Slyke (1911–1912) for amide nitrogen determination.

Free fatty acids.—Determinations were made only on the whole wheat flour and on the wheat kernels. The kernels were first ground to a fine powder in a small hand mill. The oil was extracted with ether from 100-g. samples of the material in a special extraction apparatus, and the ether was removed by evaporation on a steam bath. The residual oil was dissolved in about 20 ml. of absolute alcohol. The alcohol was removed by evaporation. The residual oil was weighed and dissolved in 30 ml. of alcohol, which had been made neutral to phenolphthalein, and the solution was titrated with tenth-normal NaOH. The free fatty acid content is calculated in terms of oleic acid according to the formula: $0.282 \times$ ml. tenth-normal alkali $\times$ 100/grams oil extracted.

True protein.—As a measure of the extent to which degradation of the proteins occurred during the storage periods, determinations were made of the so-called "true protein value" (protein nitrogen). The determinations were made in duplicate on samples equivalent to 1 g. of the moisture-free material according to the copper hydroxide method of Stutzer (1881).

Soluble nitrogen.—Extractions of the meals and flours were made with NaCl, alcohol, and sodium salicylate. In order that all extractions of the fresh and stored samples with a given solvent could be conducted to give strictly comparable results, special care was taken to have them carried out under as uniform conditions as possible.

The NaCl extractions were conducted in the temperature-controlled room at 76°F. Ten-gram samples of the material, which had been allowed to acquire room temperature before starting the extractions, were shaken at a uniform rate in a mechanical shaker for one hour with 200 ml. of 3% NaCl solution. After centrifugation, the residue was again

similarly extracted with 50 ml. of the extractant.[2]   The combined extracts were transferred to a 300-ml. graduated flask and made to volume with the salt solution.

Extractions were made also with 3% sodium salicylate solution in the same manner as described for the NaCl extractions.

In view of the fact that a large proportion of wheat protein is soluble in dilute alcohol, 10-g. samples of the material were extracted for one-half hour with 200 ml. of 70% alcohol at room temperature.   The clear extract was made up to volume in a 200-ml. flask.

Total nitrogen was determined in aliquots of the extracts.

Amino nitrogen.—Amino nitrogen determinations were made in duplicate on 10-ml. aliquots of the fresh NaCl extracts according to the Van Slyke method.   An increase in amino nitrogen is a criterion of proteolysis

Nitrogen precipitable by trichloracetic acid.—Inasmuch as trichloracetic acid precipitates the whole protein molecule, but does not precipitate its smaller hydrolytic products, the results obtained by this determination can also be taken as a measure of the extent of protein degradation that occurred during storage.

The nitrogen precipitable by trichloracetic acid was determined in 25-ml. aliquots of the original NaCl and sodium salicylate extracts already referred to.   The quantities of 15% trichloracetic acid required to give the maximum precipitation in the white flour and the whole wheat extracts were first determined in the extracts of the fresh, unstored materials, and these quantities were subsequently used in the determinations at the end of the different storage periods.   The NaCl extracts of the white flour required 6 ml. of the precipitant per 25 ml. of the extract, and 5 ml. was used for the whole wheat flour extracts.   In the case of the sodium salicylate extracts, 6 ml. of the precipitant was added per 25-ml. aliquot of both the white flour and the whole wheat flour extracts.

The trichloracetic acid precipitates were separated by centrifugation. Nitrogen was determined in aliquots of the filtered supernatant liquids, and that in the precipitates was calculated by difference.

Digestibility.—The amount of nitrogen rendered soluble by digestion of the samples with pepsin and trypsin under strictly comparable conditions was used to follow changes in digestibility of the protein as a result of storage.

A quantity of the flour equivalent to 30 g. of the moisture-free material and 2 g. of pepsin was mixed with tenth-normal HCl to a volume of 600 ml.   The mixture, in a liter Erlenmeyer flask, was incubated for

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[2] In carrying out the extractions of the meals it was not attempted to make them exhaustive because comparative data only were desired.

TABLE I

EFFECT OF STORAGE ON THE PROTEINS OF WHITE FLOUR

(Results expressed in milligrams per 100 grams flour)

Determinations	Fresh material	Stored in closed jars										Stored in bags					
		Months at 30° F.					Months at 76° F.					Months at 30° F.			Months at 76° F.		
		1	4	7	12	24	1	4	7	12	24	7	12	24	7	12	24
		mg. per 100 g. flour										*mg. per 100 g. flour*					
Moisture	12,900	12,900	12,900	12,900	12,900	14,020[1]	12,900	12,900	12,900	12,900	12,990	14,300	14,300	14,310	11,300	11,300	11,340
Total nitrogen	1,910	1,900	1,890	1,900	1,900	1,890	1,910	1,890	19,100	1,900	1,900	1,900	1,900	1,880	1,890	1,890	1,890
True protein nitrogen	1,284	1,271	1,239	1,184	1,142	1,069	1,212	1,146	1,106	1,044	989	1,142	1,121	1,048	1,086	1,012	955
Free ammonia nitrogen	23.5	24.1	23.8	24.1	24.5	24.0	24.1	23.8	23.7	24.1	24.0	24.5	24.1	24.0	24.2	24.2	23.0
Nitrogen soluble in 3% NaCl	624	431	410	410	399	392	357	337	326	315	268	409	394	376	305	284	243
Nitrogen soluble in 70% alcohol	1,026	938	924	924	903	884	882	832	812	777	713	917	900	861	791	735	683
Nitrogen soluble in 3% sodium salicylate	—	819	787	777	756	726	798	734	725	612	611	767	725	687	704	609	551
Nitrogen in NaCl extracts precipitable by trichloracetic acid	507	339	299	273	247	227	264	219	182	152	97	246	217	190	148	108	64
Nitrogen in sodium salicylate extracts precipitable by trichloracetic acid	—	793	749	719	685	651	759	717	640	575	507	695	640	596	600	499	437
Amino nitrogen in NaCl extracts	59.6	51.6	50.7	59.5	64.5	67.0	74.8	71.7	74.4	76.7	75.0	61.9	68.6	71.0	78.3	79.5	83.0
Digestibility	1,894	1,870	1,819	1,796	1,738	1,662	1,315	1,781	1,760	1,691	1,573	1,767	1,724	1,631	1,731	1,681	1,524

[1] High moisture caused by loosened cover.

72 hours at 38°C.   At the end of the period the digestion mixture was brought to pH 8–9 by addition of 16 ml. of fifth-normal NaOH.   Two grams of trypsin [3] was then added and the digestion was continued for 72 hours.   During both the peptic and tryptic digestions the digests were covered with a layer of toluene as a preservative, and the mouths of the flasks were plugged with cotton.   At the end of the tryptic digestion the mixture was filtered on folded filters.   Ten-milliliter aliquots of the filtrates were taken for total nitrogen determinations.   Corrections were made for the nitrogen in the enzymes used.

Discussion of Results

Throughout the two years' storage period all the samples remained free from any indication of insect infestation or mold.   There was no noticeable change in color or odor in the material stored in Mason jars, nor in the white flour stored in bags at 76°F.   The whole wheat flour stored in bags at 76°F. had developed by the end of two years a slightly rancid odor.   The white flour and the whole wheat flour stored in bags in the cold storage room had acquired a slight off-odor due to odors absorbed from other material stored in the room.

Tables I, II, and III show, respectively, the results of analyses of white flour, whole wheat flour, and wheat kernels made at the time they were placed in storage, and at the end of the storage intervals. These results are expressed as milligrams per 100 g. of the samples stored, and are calculated to a moisture-free basis.   For convenience in visualizing the extent of the changes, and for the sake of comparison, the changes in the proteins of the white flour and of whole wheat proteins are summarized in Table IV.   The changes are expressed as percentage decreases from the values obtained for the controls or fresh material. The percentages have been calculated from the data given in Tables I, II, and III.   In order to save space, percentage decreases during only the first and twenty-fourth months are shown.

The results show that marked alterations occurred in the proteins of the samples during storage.   These changes are manifested by decreases in solubility, in true protein content, in amount of protein precipitable by trichloracetic acid, and in digestibility.   The amino nitrogen content increased.   The extent of the changes depends on temperature, type of containers, duration of storage, and nature of the material stored. Samples stored at 76°F. were affected much more than those stored at 30°F., and those in bags more than those in sealed jars.   The changes in the white flour were in general much greater than those in the whole

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[3] Fairchild Brothers and Foster's commercial preparations of pepsin and trypsin were used for the digestions.

TABLE II

EFFECT OF STORAGE ON THE PROTEINS OF WHOLE WHEAT FLOUR

(Results expressed in milligrams per 100 grams flour)

Determinations	Fresh material	Stored in closed jars										Stored in bags					
		Months at 30° F.					Months at 76° F.					Months at 30° F.			Months at 76° F.		
		1	4	7	12	24	1	4	7	12	24	7	12	24	7	12	24
		mg. per 100 g. flour					*mg. per 100 g. flour*					*mg. per 100 g. flour*			*mg. per 100 g. flour*		
Moisture..........	10,950	10,960	10,950	10,960	10,960	10,980	10,950	10,950	10,940	10,950	10,960	14,080	14,090	14,110	10,490	10,490	10,510
Total nitrogen......	2,140	2,130	2,140	2,130	2,130	2,130	2,150	2,140	2,130	2,130	2,130	2,130	2,120	2,120	2,130	2,130	2,140
True protein nitrogen...	1,682	1,635	1,608	1,569	1,534	1,507	1,583	1,512	1,450	1,401	1,361	1,534	1,506	1,425	1,436	1,366	1,285
Free ammonia nitrogen.....	37.0	37.0	37.0	37.0	38.0	37.0	37.0	37.0	37.0	37.0	37.0	36.0	37.0	37.0	37.0	37.0	38.0
Nitrogen soluble in 3% NaCl..	756	641	587	583	572	550	588	546	536	520	477	573	546	515	509	489	428
Nitrogen soluble in 70% alcohol.....	910	742	713	707	700	689	714	679	658	637	583	686	672	653	644	595	538
Nitrogen soluble in 3% sodium salicylate..	—	1,103	1,059	1,019	998	961	1,103	1,008	977	954	894	998	956	905	956	893	866
Nitrogen in NaCl extracts precipitable by trichloracetic acid...	616	502	411	388	364	326	450	351	303	262	213	371	325	278	251	205	138
Nitrogen in sodium salicylate extracts precipitable by trichloracetic acid..	—	999	943	869	829	781	1,025	865	795	746	675	842	780	709	767	678	627
Amino nitrogen in NaCl extracts...	109.0	94.16	92.8	100.0	108.0	109.0	125.0	122.0	128.0	129.0	130.0	111.0	117.0	117.0	136.0	139.0	137.0
Digestibility.........	2,050	2,035	2,046	2,026	1,968	1,887	2,093	2,076	2,026	1,961	1,857	1,954	1,911	1,788	1,940	1,861	1,751

wheat flour.   Significant changes occurred also in the wheat kernels, although not so pronounced as in the flours.   Doubtless even greater changes than those here recorded would have been indicated had it been possible to analyze the flours immediately after milling.   Instead, there was an interval of about 10 days between the time of milling and that of the first analyses of the material.   The changes that occurred during the first month of storage were in some instances as much as three-fourths of that found at the end of two years' storage. .

Moisture.—The moisture content of the samples remained practically constant throughout the entire storage period.   The relatively constant temperature and humidity under which the materials were stored did not allow much change in moisture content.

Total nitrogen.—There was no significant change in the nitrogen content of the samples.   This was to be expected, inasmuch as there were no indications of gross spoilage.   A decrease in nitrogen content would require a much more drastic and far-reaching decomposition of the protein than would be likely to occur as a result of enzymatic or oxidative effects under the conditions to which the materials used in this study were stored.

Free ammonia.—The values found for free ammonia nitrogen were also practically constant at all intervals of the storage period.

Solubility changes.—Two striking types of change are revealed by the results of the storage studies.   One consists of a denaturation of the proteins whereby they became progressively less soluble as the length of time of storage increased.   The other change involves a breaking down of the protein-yielding products having properties that are not characteristic of native or intact protein, such as precipitability with trichloracetic acid and behavior toward the Stutzer method used for determining true protein.   The extent of denaturation was followed by determination of solubility in 3% NaCl solution, 70% alcohol, and 3% sodium salicylate.

The greatest decrease was found in solubility of the proteins of white flour in NaCl.   When stored in a bag at 76°F. for two years the percentage decrease amounted to 61%, and 57% after similar storage in a Mason jar.   The rapid rate of denaturation is also notable.   Stored in a jar under the same conditions the decrease at the end of the first month was 43% or three-fourths as much as at the end of two years.   Even storage at 30°F. resulted in denaturation to a noteworthy degree.   After storage in a jar at 30°C. for 24 months the solubility of the white flour proteins had decreased 37% of that of the fresh flour, and 40% after storage in a bag at the same temperature.

Solubility of the white flour proteins in 70% alcohol also decreased with increasing time of storage, but only to about one-half the extent it did in NaCl.   After 24 months' storage in a bag at 76°F. the amount of

TABLE III

Effect of Storage on the Proteins of Wheat Kernels
(Results expressed in milligrams per 100 grams wheat)

Determinations	Fresh material	Stored in jars			
		Months at 30° F.		Months at 76° F.	
		9	24	9	24
Moisture	10,950	10,960	10,960	10,950	10,940
Total nitrogen	2,140	2,140	2,140	2,140	2,140
True protein nitrogen	1,682	1,653	1,574	1,632	1,519
Free ammonia nitrogen	37.0	37.0	37.0	38.0	38.0
Nitrogen soluble in 3% NaCl	756	620	606	557	516
Nitrogen soluble in 70% alcohol........	910	770	748	722	713
Nitrogen soluble in 3% sodium salicylate.	—	1,061	1,038	998	922
Nitrogen in NaCl precipitable by trichloracetic acid........	616	518	509	134	163
Nitrogen in sodium salicylate precipitable by trichloracetic acid...	—	943	919	874	788
Amino nitrogen in NaCl extracts..	109.0	102.0	100.0	94.0	88.0
Nitrogen soluble in peptic-tryptic digests	2,050	2,033	1,987	2,026	1,878

nitrogen extractable with alcohol had decreased 36% of that extractable from the flour when fresh, and 19% after a similar storage at 30°F. When stored in jars the corresponding decreases in solubility were 33% and 17%, respectively. The solubility in alcohol decreased much more rapidly during the early part of the storage than later. After one month's storage in a jar at 76°F. the solubility had decreased 17%, or about one-half as much as it did during storage for 24 months.

When sodium salicylate was used as an extractant, the same decreasing trend in solubility of the proteins of the white flour on storage was shown as when NaCl and alcohol were used. Unfortunately, sodium salicylate was not used on the fresh material. The first extractions were made on the flour samples in jars after they had been stored for one month, and after seven months on the samples stored in bags. Doubtless denaturation had already occurred to a large extent before the first extractions were made.

A comparison of the solubility changes in the white flour with those in the whole wheat flour under similar conditions of storage brings out several interesting facts. The effect of storage upon the solubility of the white flour proteins, as measured by the NaCl-extractable nitrogen, was consistently much greater than the effect upon the proteins of whole wheat flour. The decreases in solubility ranged from one and one-half times to twice as much for the white flour as for the whole wheat flour. If we assume that the denaturation of the proteins on storage is an oxidizing effect, and there is reason to believe that it is, the difference in

TABLE IV

EFFECT OF STORAGE ON THE PROTEINS OF WHITE FLOUR,
WHOLE WHEAT FLOUR AND WHEAT KERNELS

(Results expressed in percentage decreases)

Determinations	Months at 30° F.		Months at 76° F.	
	1	24	1	24

WHITE FLOUR STORED IN JARS				
	%	%	%	%
True protein nitrogen..	1	17	5	23
Nitrogen soluble in 3% NaCl	31	37	43	57
Nitrogen soluble in 70% alcohol	12	17	17	33
Digestibility..............	1.3	12.0	4.0	17.0

WHOLE WHEAT FLOUR STORED IN JARS				
True protein nitrogen... ...	3	10	6	19
Nitrogen soluble in 3% NaCl	15	27	22	37
Nitrogen soluble in 70% alcohol	18	24	21	36
Digestibility......	1.0	7.0	2.0	9.0

WHEAT KERNELS STORED IN JARS				
	9	24	9	24
True protein nitrogen.......	1.7	6.4	2.9	9.69
Nitrogen soluble in 3% NaCl	18.0	19.84	26.3	31.7
Nitrogen soluble in 70% alcohol...........	15.4	17.8	20.6	21.6
Digestibility...	0.83	3.07	1.66	8.39

WHITE FLOUR STORED IN BAGS				
	7	24	7	24
True protein nitrogen..... .	11	18	15	26
Nitrogen soluble in 3% NaCl	34	40	51	61
Nitrogen soluble in 70% alcohol.................	14	19	26	36
Digestibility..............	7.0	14.0	9.0	20.0

WHOLE WHEAT FLOUR STORED IN BAGS				
	9	15	15	24
True protein nitrogen.......	9	15	15	24
Nitrogen soluble in 3% NaCl	24	32	33	43
Nitrogen soluble in 70% alcohol.................	27	28	29	41
Digestibility..............	5.0	13.0	5.0	15.0

the solubility behavior of the proteins of the two flours toward NaCl may be quite satisfactorily explained. Whole wheat flour contains to a much greater extent than white flour a number of constituents which, because of their antioxidant properties, may well protect the proteins from oxidative denaturation. These substances, residing chiefly in the wheat embryo and in the bran, represent phosphatides, unsaturated fatty acids, sterols, glutathione, and other substances that are very susceptible to oxidation. According to Rewald (1936), 80% of the phosphatides of wheat germ oil is of the lecithin type and 20% of the cephalin type. The antioxidant property of lecithin has been shown by Evans (1935) to be almost as great as that of KCN. Oils to which as little as 0.5% lecithin had been added withstood oxidation for four months at room temperature. Cephalin is readily oxidized by atmospheric oxygen at ordinary temperature (Page and Bülow, 1931). According to Sullivan and Bailey (1936a), the unsaturated fatty acids of wheat germ oil amount to 84% of the total fatty acids, and consist of α- and β-linolenic acids (3.55%), α- and β-linolic acids (52.31%), and oleic acid (28.14% by difference). Furthermore, wheat is known to contain several unsaturated sterols, the α_1- and α_2-sitosterols, and dihydroxysitosterol, in which the bran and the embryo are particularly rich. Approximately 70% of the unsaponifiable fraction of wheat embryo consists of a mixture of sterols (Sullivan and Bailey, 1936b). In addition to the sitosterols and dihydroxysitosterol there is evidence showing the presence of another unsaturated sterol with at least two double bonds. The presence of polyene hydrocarbons was also indicated. That fat constituents may be an important factor in protecting the proteins from denaturation is further supported by the fact that fat-free, solvent-extracted soybean meal shows a greater decrease in NaCl extractable nitrogen than meal containing 11% of fat.[4]

When the extent of denaturation of the proteins of the two flours is measured by the amount of nitrogen extracted by 70% alcohol, we find that the order is reversed from what it is when NaCl is used. Extraction with alcohol showed that denaturation occurs consistently to a greater extent in the proteins of whole wheat flour than in the white flour. In order to seek an explanation of this difference in behavior with respect to the two extractants, consideration must be given to the differences in the protein composition of the different parts of the wheat kernel. The white flour is derived almost entirely from the endosperm. Its proteins consist chiefly in about equal quantities of gliadin, the alcohol-soluble protein, and glutenin, a protein which is insoluble in water, neutral salt solutions, or alcohol, but is soluble in dilute acids or alkalies.

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[4] Unpublished results obtained in this laboratory.

The proteins of the endosperm comprise about 73% of the total seed protein. In addition to the endosperm, the whole wheat flour includes the bran and the embryo which represent, respectively, 22% and 4.5% of the total seed protein. The proteins of the bran (Jones and Gersdorff, 1923) consist of an alcohol-soluble protein, a globulin, and an albumin. The alcohol-soluble protein predominates. The embryo proteins consist of a globulin, albumin, and a proteose (Osborne and Mendel, 1919). Undoubtedly most of the protein extracted from white flour by alcohol represents gliadin.

The values given in Table I show that alcohol extracted 56% of the total nitrogen of the white flour, which corresponds roughly to the gliadin nitrogen. In the case of the whole wheat flour, the quantity of total nitrogen extracted was greater than that extracted from the white flour because of the higher nitrogen content of the whole wheat flour. The percentage of the total nitrogen extracted (42%) was considerably less, however. In addition to the gliadin and glutenin of the endosperm, the whole wheat contains the proteins of the bran and embryo. It appears that some, at least, of these additional proteins denatured more readily than the gliadin.

That gliadin denatured to a much less extent than other proteins of wheat is also shown (Table IV) by the fact that the percentage decrease in solubility of the white flour proteins at the end of successive storage intervals was found to be approximately twice as much in NaCl as in alcohol.

A comparison of the solubility of the proteins of whole wheat flour in NaCl with that in alcohol after different periods of storage shows consistently a little greater denaturation when measured by the NaCl extractable nitrogen, but the difference is not very significant.

Protein degradation.—The determinations of true protein nitrogen, nitrogen precipitable by trichloracetic acid, and amino nitrogen were designed to show whether any degradation of the proteins occurred on storage.

In the true-protein determinations the insoluble copper compound of the coagulated protein represents the unchanged native protein. The degradation products, such as peptides, proteoses, and amino acids, remain in the filtrates. The results show consistently decreasing values in the true protein nitrogen content of the flours as the storage intervals increased. The decreases were significantly greater in the white flour than in the whole wheat flour, and greatest in the samples stored in bags at 76°F. Even at 30°F. the white flour stored in jars for 24 months showed a percentage decrease of 17%. When stored in bags at 76°F. the decrease amounted to 26%. These decreases show definitely that the protein had undergone some kind of breakdown during storage.

Trichloracetic acid precipitations made on the NaCl extracts of the stored samples also show a breakdown of the proteins, increasing with the length of the storage periods.   It is emphasized, however, that the figures indicate a greater extent of protein breakdown during storage than actually occurred.   Consideration must be given to the diminishing amounts of protein present in the NaCl extracts with increasing storage periods as a result of denaturation of the proteins in the flour.   Thus, for example, in the case of the white flour stored in a bag at 76°F. for two years the amount of nitrogen precipitated by trichloracetic acid decreased from 507 mg. to 64 mg., a drop representing 87%.   If, however, the decrease is calculated on the amount of nitrogen remaining in the NaCl extract after precipitation with trichloracetic acid rather than on the amount precipitated, thus largely avoiding in the calculation the complicating factor of denaturation, the much smaller decrease of 53% is indicated.

The amounts of amino nitrogen found in the NaCl extracts of the flours show a definitely increasing trend with the length of storage periods.   At the end of 24 months the amino nitrogen found in the salt extracts of the white flour stored in jars at 30°F. had increased from 59.6 mg. to 67 mg., and to 75 mg. when stored at 76°.   The sample stored for the same periods in a bag at 30°F. showed an increase of amino nitrogen to 71 mg., and to 83 mg. when stored at 76°F.   The corresponding increases for whole wheat flour were not as great, particularly at low-temperature storage.   At 76°F., however, the sample stored in a bag showed an amino nitrogen increase at the end of 24 months from 109 mg. to 137 mg.   For the reason given below the actual increases in amino nitrogen must be materially greater than the amounts found in the extracts.

As in the case of the trichloracetic acid precipitations, an exact evaluation of the amino nitrogen results obtained is complicated by the fact that the NaCl extracts of the samples after each successive storage period contained decreasing amounts of protein.   It has been shown that proteins yield amino nitrogen in amounts corresponding very closely to one-half of their lysine nitrogen; consequently there should be added to the amounts of amino nitrogen actually found in the extracts of the stored samples the amounts of amino nitrogen that would have been yielded by the proteins rendered insoluble during the storage periods. The results would then show materially larger increases of amino nitrogen than those actually determined (Table I).   No data, however, are available on the exact amount of amino nitrogen represented by the denatured protein fractions.   An approximation can be made by assuming that the free amino nitrogen of the denatured proteins represents 5% of their total nitrogen.   This assumption is based on the fact that

certain proteins, such as casein and hemoglobin, yield from 5% to 6% of free amino nitrogen (Van Slyke and Birchard, 1913–1914). It is realized that 5% may be somewhat too high for the white flour because of the low lysine content of gliadin. On the other hand, glutenin, the other protein of white flour, contains a relatively large amount of lysine.

If the data for amino nitrogen in the NaCl extracts shown in Table I were recalculated on the basis outlined above, the results would indicate that there is a very material increase in the amino nitrogen of the samples as a result of storage. The amount of nitrogen rendered insoluble by the end of any given storage period is equal to the total nitrogen in the extract minus the total nitrogen found in the salt extract of the fresh material. Five percent of this nitrogen added to the amount of amino nitrogen actually found in the NaCl extracts will give a more nearly accurate figure for amino nitrogen increase. As an illustration, the amino nitrogen found in the NaCl extract of white flour stored for 24 months in a bag at 76°F. calculated on the basis above outlined becomes 102 mg. instead of 83 mg., which represents an increase of 71% as compared with the amino nitrogen in the salt extract of the fresh material.

It is of interest to point out that there is an apparent inconsistency in the amino nitrogen values determined at the end of one and four months' storage (Table I). The amounts of amino nitrogen actually found in the salt extracts were less at the end of the first and the fourth month than that of the fresh material. These apparent decreases are accounted for by the higher rate of denaturation of the proteins during the first few months than subsequently, and they disappear when the values have been corrected for the amino nitrogen in the denatured fractions.   .

Digestibility.—The data presented for the digestibility values represent the amount of soluble nitrogen found in the peptic-tryptic digests of the samples at the end of the various storage periods.

The results of the digestibility determinations indicate a definitely decreasing trend in the digestibility of the proteins as the periods of storage increased. The decreases in digestibility were greater in the white flour than in the whole wheat flour, and least in the kernels. The decreases during the first seven months, however, were small compared with the changes already noted in solubility, true protein value, and precipitability with trichloracetic acid for the same periods. At the end of 24 months the white flour stored in bags at 76°F. showed a decrease of 20% compared with 15% for whole wheat flour. Wheat kernels stored in jars for 24 months showed a digestibility decrease of 8%. For some reason that we cannot explain, there was a slight increase in

the values found for whole wheat flour stored in jars at 76°F. at the end of the first and fourth months.

Free fatty acids.—It is well known that on aging cereal flours develop increasing amounts of free fatty acids. Zeleny and Coleman (1938, 1939) recently have contributed to our knowledge in this field, and have surveyed the literature on the subject.

Kozmin (1935) and Barton-Wright (1938) reported that deterioration of gluten quality during storage is to be attributed largely to the effect of accumulating quantities of free fatty acids.

In order to get some collateral data that might be of value in explaining the nature of the causative agents that bring about the changes in the proteins during storage, determinations were made also on the free fatty acid content of the samples at the end of the storage intervals. Because of the small quantities of fat in the white flour, these determinations were made only on the whole wheat flour and on the kernels.

The rate of increase in free fatty acids in the whole wheat flour during storage varied with the temperature, type of container, and duration of storage up to the end of 12 months. There was not much increase during the second year. At the time the flour was placed in storage the fatty acid content amounted to 7% of the extracted oil. After storage for 12 months in a jar at 30°F. the value had risen to 13%, and to 35% when stored at 76°F. When stored in bags under otherwise the same conditions the values had increased to 15% and 36%, respectively. The wheat kernels gave a value of 12% and 15% after storage in jars for two years at 30° and 76°F., respectively.

Possible Causes of Storage Changes

The question naturally arises as to what may be the agents that are accountable for the protein changes observed. Are they attributable to enzymatic, oxidative, or other effects? That they were due to the action of molds or insects seems to be pretty well excluded. We have no reason to believe that they can be explained by bacterial action. The degradation of the proteins as manifested by increase in amino nitrogen, decrease in true protein, and decrease in the amount of protein precipitated by trichloracetic acid, we believe is best explained by the action of enzymes. It certainly seems that the increase in amino nitrogen can be accounted for only by enzymatic effect whereby cleavage of the protein occurs at the peptide linkages. It is known that wheat contains enzymes that attack proteins. Balls and Hale (1935, 1938) identified the proteinase of whole wheat and wheat bran as belonging to the papain type of enzymes. More recently, Hale (1939) extracted a proteinase from patent flour that appeared to be the same as that in wheat bran. The proteinase

showed a definite, but not extensive, proteolytic activity, indicating that it may be accompanied by a peptidase. Its effect is regarded as a " protein modification rather than extended hydrolysis."

Decrease in solubility of the proteins might be ascribed, at first thought, to the effect of free fatty acids. This explanation, however, finds no support in the fact that denaturation as measured by solubility in NaCl occurred to a greater extent in white flour than in whole wheat flour which contained much more fatty acids. The most plausible explanation is that which has been already discussed on another page, namely, that the denaturation was the result of oxidation, occurring spontaneously or as the effect of oxidases present in the flours. Further work is planned in order to get more evidence on this question. Feeding experiments are in progress to study the effect of storage on the nutritional value of the proteins.

Summary

The effect of storage under different conditions upon the proteins of white flour, whole wheat flour, and wheat kernels has been studied at various intervals over a period of two years. The results show that three different types of alterations occur: (1) a decrease in the solubility of the proteins, (2) a partial breakdown of the proteins indicated by decrease in true protein content, by decrease in the amount of nitrogen precipitable by trichloracetic acid, and by increase in amino nitrogen, and (3) decrease in digestibility.

The extent of the changes depends on temperature, type of containers, duration of storage, and the nature of the material stored. Samples stored at 76°F. were affected more than those stored at 30°F., and those in bags more than those in sealed jars. Changes in white flour were in general greater than in whole wheat flour. Significant changes occurred also in the wheat kernels, although not as great as in the flours. The total nitrogen and free ammonia remained unchanged. The extent of denaturation of the proteins was followed by determination of solubility in 3% NaCl, 70% alcohol, and 3% sodium salicylate. The greatest decrease was found in solubility of the proteins of white flour in NaCl. When stored in a bag at 76°F. for two years the percentage decrease amounted to 61%, and 57% after similar storage in a sealed glass jar. The alteration of the proteins is ascribed to the effect of enzymes and oxidation.

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OBSERVATIONS ON THE CHARACTER OF RECORDING-DOUGH-MIXER CURVES ON FLOURS DILUTED WITH WHEAT STARCH

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Effort toward a determination of the correlation between physical properties of doughs and baking performance has been continued during the past decade. A number of people have done a considerable amount of work but comparatively little has been published.

One of the obstacles in comparing flours is the variation resulting from differences of protein level. Larmour, Working, and Ofelt (1939) showed that recording dough-mixer curves for any given variety varied in character at different protein levels and that the height of the curve increased with increasingly higher protein content. In order to minimize differences in protein quantity as a factor in studying baking properties of wheat flours, Sandstedt and Ofelt (1940) used flours diluted with starch to a common protein level. This suggested that the mixing characteristics of flours also should be compared on a common protein basis. An investigation was made of the effects of dilution, by the addition of wheat starch, on a number of flours. This paper is a report of the observations made.

Methods and Materials

The wheat starch used for dilution purposes was prepared according to the technique used by Sandstedt, Jolitz, and Blish (1939), discarding the "amylo-dextrin" fraction.

The several variety-protein series of flours used by Sandstedt and Ofelt (1940) for the study of baking properties were also used in this investigation. These samples were obtained from the Department of Milling Industry at Kansas State College, through the courtesy of Dr. E. G. Bayfield and were similar to the series used by Larmour, Working, and Ofelt (1939) in their investigations on quality of hard winter wheats. The southwestern composite was made up of experimentally milled flours from 18 southwestern wheats. The Cheyenne selection was obtained from the Department of Agronomy of the University of Nebraska, and the Chiefkan was a field sample.

A National Micro Recording Mixer was used and each curve represents 35 g. of flour on a 15% moisture basis, with normal baking absorption. Absorption was determined on each of a number of normal flours and on definite dilutions of these flours with wheat starch.
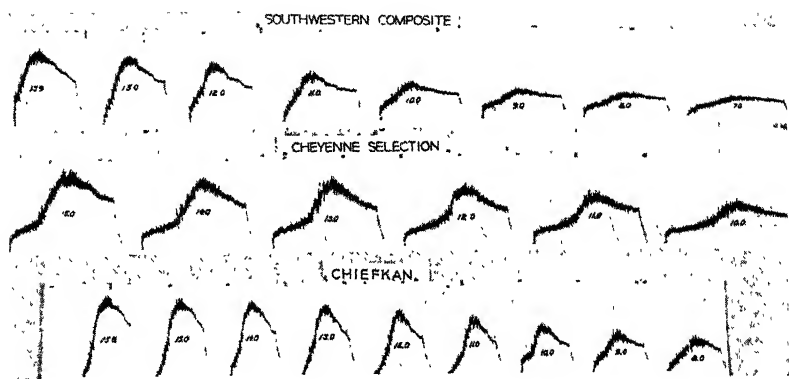
Fig. 1. Recording-micro-mixer curves that show the effects on curve character obtained by diluting with wheat starch to different protein levels. The curve on the extreme left in each series is

From information obtained in this manner it was possible to calculate the absorption attributable to the starch and thus to calculate the proper absorption for any dilution of any flour of known absorption. On a 15% moisture basis the starch used for these investigations had an absorption of 53%.

Experimental

A composite of many southwestern flours was chosen for this study because of its representative character, and the samples of Chiefkan and Cheyenne selection were chosen because of their divergent characteristics. Curves were run on these flours at their normal protein levels and on mixtures of these flours with wheat starch added in amounts calculated to reduce the protein content in decrements of one percent. These curves are shown in Figure 1. The curves are similar to those obtained by Larmour, Working, and Ofelt (1939) on a series of normal flours at different protein levels, and within the normal protein ranges could easily be substituted for a natural series. These curves do not show the decrease in time to minimum mobility with increasing dilution with starch, as was reported by Markley (1938); on the contrary, the curves show an increase to minimum mobility with increasing dilution. This difference in results is probably due to the difference in the absorptions used in the two investigations; in this investigation the predetermined baking absorption was used, while in Markley's investigation the absorption was so adjusted as to give a minimum mobility of 550 Brabender units; that is, the absorption was adjusted to make all curves the same height. Merritt and Bailey (1939) found that this absorption, which gives a minimum mobility of 550 Brabender units, does not correspond to baking
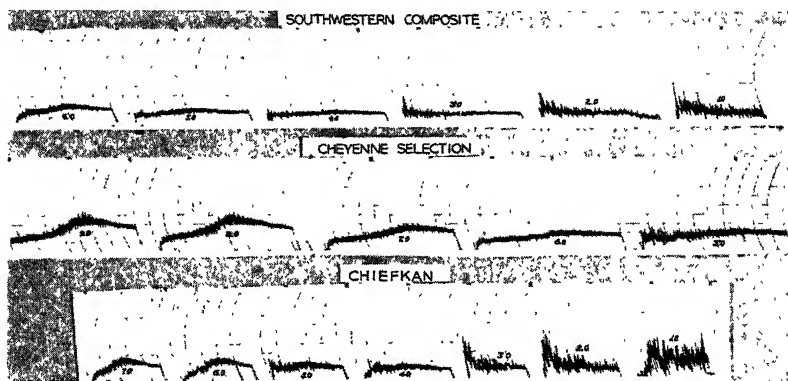
that of the normal flour at the original protein level.   The following curves are at dilutions calculated to represent 1% decrements in protein content.   The numerical values represent the protein level of the flours and of the flour-starch mixtures.

absorption.   It would require considerable readjustment in absorption to bring all the curves in each series shown in Figure 1 to the same height.

That adjusting absorptions to bring all curves to a common height would give an entirely different series of curves from those shown in Figure 1 is shown in Figure 2.   This series of curves was run on a 14.8% protein hard winter wheat flour at the naturally occurring protein level and diluted with starch to 9%, 7%, and 6% protein levels.   The upper row of curves is for the original flour and for the different dilutions of the flour, each given normal baking absorption, while the curves immediately below are for the same dilutions given absorptions which would bring all curves to a 6-cm. height.   It was impossible to bring the curves for mixtures with less than about 6% protein to this particular height, since there is of course a lower limit to the amount of water that may be used to produce a dough. The wide fluctuations in the 6% protein curve are indicative of the dryness of the dough.   These curves, of course, do not resemble curves that would be obtained with the Brabender farinograph, but the same tendencies would be evident regardless of the particular shape of the curve.

An observation of interest was that the flour-starch mixtures that were given normal baking absorptions seemed to retain true flour-dough character down to and including those at a 4% protein level. At a level of 3% protein or less the mixtures had starch characteristics; they would not "pick up" in the mixer and did not form the smooth continuous dough characteristic of wheat flours.   It should be noted (Fig. 1) that there is an indication of change in the type of curve in each series between the 5% and 6% protein levels.

Curves were run on the original flours at the naturally occurring protein levels in each of the variety-protein series. The curves for these flours are shown in row *A* for each variety in Figure 3. These curves show the same approximate inter- and intra-varietal differences as did curves run by Larmour, Working, and Ofelt (1939) on a similar series of flours from the previous crop year. It should be noted that protein content is not correlated with dough development time; this is not in agreement with Markley, Bailey, and Harrington (1939), again probably as a result of the different methods of determining absorption.

All of the flours in each of the variety-protein series were diluted to a 9% protein level by the addition of wheat starch and curves were run on the resulting flour-starch mixtures. The resulting curves are
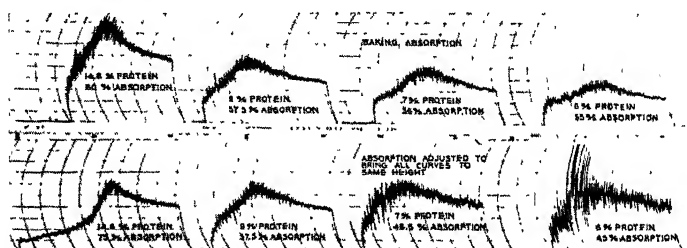
Fig. 2. Curve characteristics of a flour and its dilutions with starch as affected by the use of baking absorption (above) in contrast to absorption adjusted to bring all curves to the same height (below).

shown in row *B* for each variety in Figures 3. The curves for the undiluted flours indicate that the height of the curve is dependent upon the protein content of the flour. Similarly all flours diluted to a constant protein level within a given variety produce curves of a nearly constant height. The heights of curves of the diluted samples from all varieties, with the exception of Chiefkan, Early Blackhull, and Blackhull, were similar. Curves from the diluted samples of Chiefkan and Blackhull were higher at the low protein levels than those from the other varieties and decreased in height with increase in protein content of the original flour used in the flour-starch dilution mixture. The curves for the diluted samples of Early Blackhull were also higher than those from varieties usually considered more desirable but did not exhibit the decline in height shown by the Chiefkan and Blackhull varieties. It should be noted that, while curve height was apparently governed to a large extent by protein content, the curve for the diluted flour retained much of the character or type of the curve obtained from the original flour, and that the curve of the diluted flour varies with the naturally occurring protein level. Sandstedt and
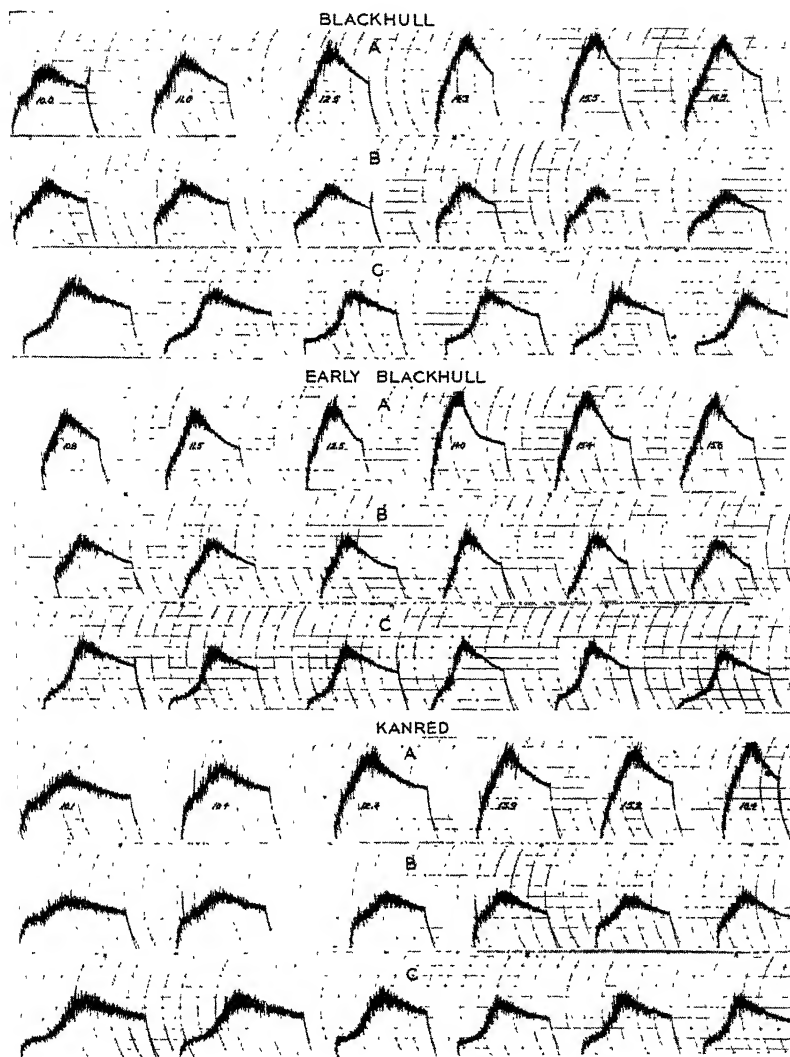
Fig. 3. Recording-micro-mixer curves on flours milled from different varieties of wheat. Each variety is represented at different naturally occurring protein levels and by flour-starch mixtures made from these flours. Curves in series A are on the original flours at their indicated protein levels. All curves in series B are on flour-starch mixtures obtained by diluting the flour directly above it (in series A) to a 9% protein level by addition of wheat starch. Curves in series C correspond to those directly above in series B, with the exception that all baking ingredients were added.

Fig. 3. Recording-micro-mixer curves on flours milled from different varieties of wheat. Each variety is represented at different naturally occurring protein levels and by flour-starch mixtures made from these flours. Curves in series A are on the original flours at their indicated protein levels. All curves in series B are on flour-starch mixtures obtained by diluting the flour directly above it (in series A) to a 9% protein level by addition of wheat starch. Curves in series C correspond to those directly above in series B, with the exception that all baking ingredients were added.
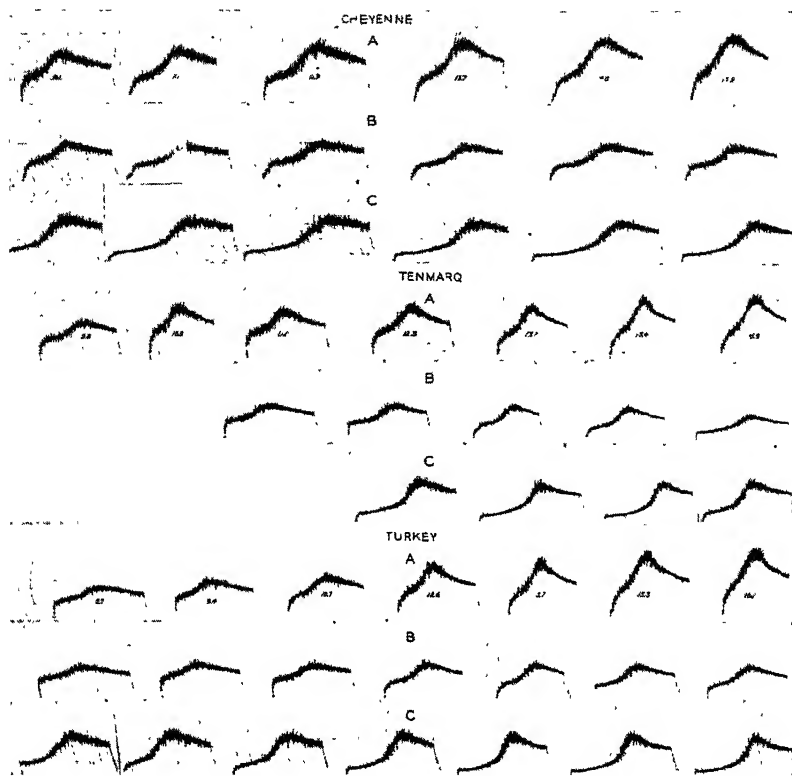
FIG. 3.—Concluded

Ofelt (1940) have indicated that baking quality of diluted flours varies in a similar manner.

As a matter of interest, curves were run on the flour-starch dilution mixtures with the addition of baking ingredients. These included sugar 6%, salt 1.75%, shortening 3%, yeast 2%, and dry milk solids 6%. The resulting curves are shown in row *C* for each variety in Figure 3. The same comments with regard to the height and character apply to these curves as to the curves run on the flour-starch dilution mixtures with the simple addition of water.

Summary

Three flours of widely divergent type were used in conjunction with wheat starch to produce flour-starch mixtures with protein levels reduced in decrements of one percent. Recording-mixer curves were obtained from these mixtures at predetermined baking absorptions. The curves were similar to curves obtained from flours with the same

naturally occurring protein content; they showed the same tendency to decrease in height and flatten out with decrease in protein. These curves did not show a decrease in time to minimum mobility with increasing dilution.

Recording-mixer curves were obtained on flours from nine varieties of wheat, each variety being represented by samples at different protein levels. Each of these flours was reduced to a 9% protein level by dilution with wheat starch.

Recording-mixer curves were obtained on these flour-starch mixtures with the addition of water and also with the addition of the baking ingredients normally used in commercial practice. The curves, on both the natural and the diluted flours, show that the height of the curve is largely determined by the protein content and that the time to minimum mobility is not correlated with protein content, *i.e.*, that mixing time is not dependent on protein content but is largely a varietal characteristic. The curve for each of the diluted flours retained much of the character of the original flour. The series of curves for Chiefkan, Blackhull, and Early Blackhull indicate that the protein content is not the only factor determining curve height.

Attention is drawn to the differences in interpretation of curves, which is occasioned by so adjusting absorption as to bring all curves to the same height in contrast to using baking absorption.

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THE LIPIDS OF CORN STARCH [1]

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The lipids of corn starch that cannot be removed by the usual fat solvents make up about one-half of one percent of the commercial starch. This material is liberated upon acid hydrolysis of the starch and appears in the hydrolysate as an insoluble residue. Schoch (1938) has reported that the fatty acids from cereal starches can be removed by Soxhlet extraction with water-miscible fat solvents such as methanol, the cellosolves, or 80% dioxane in water.

Taylor and Nelson (1920) were the first to make a study of the lipids associated with starch. They found that, on acid hydrolysis of purified corn starch, fatty acids were liberated amounting to 0.61% of the dry weight of the starch. They reported that palmitic acid was the principal constituent, and that an unsaturated substance of unknown structure was present. They believed that the palmitic acid was attached only indirectly to the carbohydrate, but directly to the unsaturated component present. Taylor and Lehrman (1926) found the fatty acids obtained from corn starch to be composed of 40% oleic, 36% linolic, and 24% palmitic. Taylor and Iddles (1926) found that only the alpha portion of corn starch contained fatty acids. Rask and Phelps (1925) extracted corn starch with a mixture of ethyl alcohol, concentrated ammonia, and water, and obtained 0.54% "lipoid material" without hydrolyzing or gelatinizing the starch. Taylor and Werntz (1927) found that the material extracted by this reagent was a mixture of fatty acids and extraneous material and that only 20% to 25% of the fatty acids present in corn starch were removed by this treatment. Taylor and Sherman (1933) found that the fatty acids were liberated preferentially during acid, diastatic, or alkaline hydrolyses of corn starch and that the unsaturated acids were most easily removed. Schoch (1938) suggested that since free fatty acids can be extracted from cereal starches with water-miscible fat-solvents, it would appear that the fatty acids were distributed throughout the starch granule as an extraneous impurity and not in chemical combination with the starch.

The present investigation was undertaken for the purpose of making a more detailed study of the lipids from corn starch than has been

made before. This paper reports the results of a study on the extraction of corn starch with methanol, and of the analysis of the lipids in this extract. The analyses of the lipid material removed from corn starch by acid and by diastatic hydrolysis are also given. The fatty acids present in the starch have been separated and identified.

Fatty Material in Starch Obtained from Corn Kernels Where the Embryos Were Removed before Milling

In the commercial milling process a portion of the starch comes into intimate contact with the embryos when the steeped corn is put through disintegrating mills to rupture the kernel, and when this macerated corn is put into tanks where the germ floats off.

In order to be certain that starch did not adsorb or pick up its fatty acid content during this process, the germs from 500 g. of Reid's Yellow Dent corn were removed from the grains before processing. The endosperms were then steeped at 50°C. for 24 hours in water containing 0.25% sulfur dioxide. The steep water was removed and the process repeated. The steeped endosperms were ground in a Buhr mill, sufficient water being added to make a final "slop" with about 60% water. The ground material was screened and the material that passed through the screen was bolted on No. 17 bolting silk (17 × ×; 163 meshes per inch). Starch was separated from gluten by suspending the mixture in water, centrifuging, and then scraping the gluten off the settled material with a spatula. The process was repeated four times and on the third centrifuging no gluten was observed on the surface of the starch cake. This purified starch was air-dried for three days and then dried in a vacuum oven at 100°C. for five hours.

The lipid content of the dry starch was determined by the acid hydrolysis procedure of Taylor and Nelson (1920), which is described below under the heading "Analysis of lipids liberated by acid hydrolysis." The final ether extract of the residue from hydrolysis was dried in vacuum at 50°C. to constant weight.

The fatty material thus liberated from the starch was found to be 0.64% on dry substance basis (as compared with 0.61% found by Taylor and Nelson for purified corn starch).

This shows that the fatty material ordinarily present in corn starch does not get on or into the starch during the milling process.

Preparation and Analysis of Factory Starch

The commercial corn starch used in this work was supplied by the Union Starch and Refining Company of Granite City, Illinois. It was produced by the wet milling process and was taken from the fourth American filter. After being air-dried in the laboratory, the moisture

content of this starch was found to be 11.5%. Analysis of the starch, calculated on a dry-substance basis, gave the following results: protein, 0.43%; ash, 0.10%; fat (by hydrolysis), 0.64%; materials extractable by ether, 0.062%; and phosphorus, 0.016%.

Extraction of Starch

While ether extraction of the products of acid hydrolysis of starch yields about 0.62% of the dry weight of the starch as ether-soluble substances, it is not possible to remove more than a small fraction of these lipids by ether extraction of the original dry starch. Analysis of the lipids obtained after acid hydrolysis must necessarily fail to give much information as to the actual state of combination characteristic of the lipids in the original starch. Whether or not the fatty acids, which make up the larger percentage of the lipids obtained after acid hydrolysis, are present in the starch as such, or in combination with glycerol (or with the starch itself), is a question of much importance. Water-soluble fat solvents have been shown to remove a fairly high fraction of the lipids present. If a complete, or nearly complete, extraction of the lipids could be obtained by using such solvents, the question as to the state of the fatty acids in the starch, before hydrolysis, could be answered by analysis of such extracts.

For the following extraction experiments, factory starch that had been dried in vacuum at 100°C. for 12 hours was used.

1. Soxhlet extraction with methanol as solvent.—Samples of dried starch (50 g. each) were placed in 42 × 120 mm. paper extraction thimbles, and extracted with absolute methanol (A. R. grade) in Soxhlet extractors for 24, 36, and 48 hours, respectively. At the end of the extraction period the methanol was distilled off and the flasks were dried in an oven at 50°C. to a constant weight (methanol extract). Ether was then added to the flasks, refluxed for 10 minutes, cooled, filtered into a tared flask, the ether removed on a steam bath and the flasks dried to constant weight (methanol extract soluble in ether). To obtain the amount of the methanol extract that was soluble in water, the original dried extract was mixed with water and allowed to stand for 24 hours with occasional stirring, then filtered and the filtrate evaporated to dryness. Kjeldahl nitrogen was determined on the methanol extract, the water-soluble portion, and the portion that was insoluble in water and ether but soluble in methanol. The fatty material was determined in the extracted starch by liberating with acid hydrolysis as described below. Results of these analyses are given in Table I.

It is seen that not all of the fatty material can be removed from the starch by the extraction method described above. Nor is all of the

material extracted by methanol of a lipid nature. This is as would be expected, since the starch contained 0.43% protein. The results indicate that some protein is peptized by the methanol, and also that some water-soluble material which is not protein is removed. (This material shows no reducing properties when tested with a small amount of Benedict's solution.)

TABLE I

ANALYSES (AS PERCENTAGES OF DRY WEIGHT OF THE STARCH) OF THE EXTRACT AND OF THE EXTRACTED STARCH OBTAINED THROUGH METHANOL EXTRACTION OF DRY STARCH IN A SOXHLET EXTRACTOR

	Time of extraction (hours)		
	24	36	48
	%	%	%
Methanol extract	0.68	0.78	0.79
Methanol extract soluble in ether	0.40	0.46	0.48
Methanol extract soluble in water	0.14	0.14	0.15
Methanol extract insoluble in ether and water	0.10	0.15	0.16
Protein in methanol extract	0.06	0.11	0.12
Protein in methanol extract soluble in water	0.02	0.02	0.02
Protein in methanol extract insoluble in ether and water	0.05	0.08	0.10
Fatty material liberated from extracted starch by acid hydrolysis	0.23	0.18	0.17

2. Soxhlet extraction with methanol as solvent on starch that had been ground in rod mill.—Since all of the fatty material was not removed by the above procedure, it was thought that, by grinding the starch in a rod mill to break the granules and then extracting with methanol, more of the lipids might be removed.

Samples of 200 g. of starch were ground for 75 and 150 hours in a rod mill that was designed by Stamberg and Bailey (1939). The mill consisted of a one-gallon ball-mill jar with 30 steel rods 13 cm. long and 2 cm. in diameter. The jar rotated at the rate of 13.0 rpm. After grinding, the starch was extracted for 48 hours as previously described. The results are given below:

Time in mill (hours)	75	150
Methanol extract (%)	0.77	0.79

The starch that had been ground for 75 hours and extracted with methanol for 48 hours was extracted for an additional 104 hours with only a 0.01% increase in extractable material.

No more material could be extracted from the ground starch than from the original. It appears, therefore, that the complete removal of the fatty substances with methanol is not dependent upon the disruption of the starch granule. In view of this finding it would seem

unlikely that the fat is distributed through the starch by simple occlusion.

3. *Soxhlet extraction with other solvents.*—Neither ethanol nor a 70/30 mixture of methanol and ether gave better results than did methanol alone.

4. *Methanol extraction in a Stokes extractor.*—In the Soxhlet extractor, the extraction is effected by cold solvent. Extraction with hot solvent was tried, employing an extractor described by Stokes (1914). The sample to be extracted was placed in a thimble, covered by a plug of fat-free cotton, and supported in the flask by means of a wire passing through the cork connecting the flask with the condenser. The thimble containing the sample was allowed to remain in contact with the boiling methanol for 12 hours and was then pulled up out of the solvent (by means of the wire) and extracted by the boiling vapors for an additional 24 hours.

TABLE II

ANALYSES (AS PERCENTAGES OF DRY WEIGHT OF STARCH) OF EXTRACT AND OF THE EXTRACTED STARCH OBTAINED AFTER HOT METHANOL EXTRACTION OF DRY STARCH IN A STOKES EXTRACTOR

	Sample (grams)			
	18.0	20.0	73.7	70.0
	%	%	%	%
Methanol extract	1.06	1.00	1.03	1.00
Methanol extract soluble in ether	0.66	0.62	0.65	0.62
Fatty material liberated from extracted starch by acid hydrolysis	0.04	0.04	0.03	0.04

The weights of the methanol extract and of the fraction of the methanol extract which was soluble in ether were determined as described above. Fatty materials remaining in the extracted starch were freed by acid hydrolysis and determined. Results are those shown in Table II.

The same procedure was carried out, using starch containing 10% moisture in place of dry starch. After the extraction was completed, sufficient ether was added to the extract to make about a 70/30 methanol-ether mixture. Anhydrous sodium sulfate was added and the mixture was shaken and allowed to stand for 24 hours. Then the sodium sulfate was filtered from the extract, broken up in a mortar, and washed three times with dry ether, the washings being added to the extract. The results are shown in Table III.

These results indicate that practically all (95%) of the lipid material can be removed from corn starch by extracting with hot methanol and that it is not necessary to use dry starch in the process. The

TABLE III

ANALYSES (AS PERCENTAGES OF DRY WEIGHT OF STARCH) OF EXTRACT AND OF THE
EXTRACTED STARCH OBTAINED AFTER HOT METHANOL EXTRACTION
OF STARCH CONTAINING 10% MOISTURE IN A STOKES EXTRACTOR

	Sample (grams)		
	70.0	70.0	70.0
	%	%	%
Methanol extract	1.00	1.05	1.08
Methanol extract soluble in ether	0.62	0.64	0.62
Fatty material liberated from extracted starch by acid hydrolysis	0.04	0.03	0.06

extracted starch retained its characteristic granular structure and its
ability to form a paste with hot water, as was also observed by Schoch
(1938).

Analysis of Lipids Obtained from Starch by Methanol
Extraction, by Acid Hydrolysis, and by Diastatic
Hydrolysis

1. Preparation of lipid samples: (a) Extracted by methanol.— A
2,100-g. sample of factory starch (1,890 g. dry substance) was ex-
tracted with methanol by Stokes' method. Sufficient ether was added
to the extract to make a 70/30 methanol ether mixture and this was
dried two days with freshly ignited sodium sulfate. After filtering,
the solvents were distilled off, under diminished pressure, in a current
of nitrogen, the temperature never exceeding 40°C. The residue was
extracted with anhydrous ether, the solution filtered, and made to
1,000 ml. The fat content of this solution, as determined on several
25-ml. aliquots, was equivalent to 0.61% of the starch (d.s. basis).

For all the lipid determinations an aliquot of the above solution was
used, the concentration being checked each time by evaporating a
larger or an equal aliquot in a tared flask. The solvent was distilled
off under diminished pressure in a current of nitrogen.

*(b) Liberated by acid hydrolysis.—*A 15,000-g. sample of factory
starch (13,500 g. dry substance) was hydrolyzed with hydrochloric
acid in essentially the same manner as described by Taylor and Nelson
(1920). Batches of the starch were suspended in four times their
volume of water. This suspension was poured into approximately
three times its volume of boiling hydrochloric acid solution (made by
adding 1 volume of concentrated hydrochloric to 2½ volumes of
water). Boiling was continued until a test sample gave no color with
iodine. The mixture was then cooled to 20°C., filtered, and the residue
was washed with cold water until the washings were free of chlorides.

After the residue and filter paper had been dried for 4 hours at 50°C. in vacuum oven, they were placed in a Soxhlet extractor and extracted with ethyl ether for 28 hours. This ether extract was made up to volume with ether and aliquots of this solution were used for the various lipid determinations. The hydrolysis was carried out in an atmosphere of nitrogen and a stream of nitrogen was passed through the vacuum oven during drying of the residues in order to prevent oxidation of the unsaturated fatty acids.

(c) *Liberated by diastatic hydrolysis.*—To obtain the lipids for this work, 1,110 g. of starch (1,000 g. dry substance) were hydrolyzed with malt extract. The hydrolysis was carried out on ten "111-gram" samples as follows:

The enzyme was obtained by stirring 100 g. of ground "malted" barley in 600 ml. of water for one hour, then centrifuging and filtering. Two ml. of this extract was used for each gram of starch.

A 111-g. sample of starch was suspended in 200 ml. of water, and added to 1,000 ml. of boiling water, with constant stirring. The mixture was boiled for 30 minutes, and then cooled to 50°C. To this, 220 ml. of malt extract was added and the mixture digested until there was no color produced when tested with iodine (usually 24 hours). During the digestion period, a stream of nitrogen was slowly bubbled through the suspension to prevent oxidation. At the end of this period, the material was cooled to about 20°C., filtered, and the residue washed until the filtrate showed no reducing power when tested with Fehling's solution. The residue was dried in a stream of nitrogen in a vacuum oven at 50°C. for 6 to 8 hours and then extracted with ether in a Soxhlet extractor for 24 hours.

The ethereal extracts were concentrated to about 700 ml., dried with anhydrous sodium sulfate, filtered, and made up to 1000 ml. with dry ether. Aliquots of this solution were used in all determinations.

2. *Lipid analysis.*—Iodine value (Wijs), acid value, saponification value, total fatty acids, and ester value were determined as described by Jamieson (1932). The thiocyanogen value was determined by the Kaufmann method as described by McKinney (1938). Saturated and unsaturated fatty acids were separated by the Twitchell (1921) procedure. The iodine numbers were determined also by the Rosenmund-Kuhnhenn (1923) method which uses pyridine sulfate dibromide as the halogenating agent. Unsaponifiable matter was determined by the Allen and Thomson method as described by Lewkowitsch (1909), and the phosphatides by Boyd's (1931) modification of Bloor's (1929) oxidation method. Saponification was carried out in an atmosphere of nitrogen in order to prevent oxidation of the unsaturated fatty acids.

3. Calculation of the unsaturated fatty acids.—The amounts of the various unsaturated fatty acids present in the total fatty acids were calculated according to the following equations of Kaufmann and Keller (1929).

$$G + Ln + L + O = 100$$
$$273.7 \, Ln + 181.1 \, L + 89.9 \, O = 100 \text{ (I.V.)}$$
$$182.5 \, Ln + 90.5 \, L + 89.9 \, O = 100 \text{ (T.V.)}$$

G = % saturated acids (from Twitchell separation)
Ln = % linolenic acid
L = % linolic acid
O = % oleic acid
I.V. = iodine value of total fatty acids (Wijs)
T.V. = thiocyanogen value of total fatty acids

The above equations can be simplified as follows:

Let:
$$\text{Unsaturated fatty acids (U.A.)} = 100 - G$$

Then:
$$O = 0.994 \, \text{U.A.} + 1.113 \; \text{T.V.} - 1.104 \; \text{I.V.}$$
$$L = 0.984 \, \text{U.A.} + 1.112 \;\; \text{I.V.} - 2.209 \; \text{T.V.}$$
$$Ln = 1.096 \; \text{T.V.} - 0.0076 \; \text{I.V.} - 0.978 \; \text{U.A.}$$

When oleic, linolic, and linolenic acids are present, the validity of the method for calculating their relative amounts depends upon the validity of the assumption that the theoretical iodine and thiocyanogen values are correct. Kass, Lundberg, and Burr (1940) have recently shown that the iodine numbers (Wijs) of pure linolic and linolenic acids agree with the theoretical values, but that the thiocyanogen values of these acids, determined according to the officially recommended methods, are 96.3 and 171 respectively. These values will vary, also, with the conditions of the determination. They suggest that empirical values be substituted for the theoretical constants in the equations for the calculation of the amounts of the unsaturated fatty acids. However, the equations given above have been employed in arriving at the distribution of unsaturated acids reported in this paper.

In most cases where a mixture of saturated acids, oleic, linolic, and linolenic acids are present, the use of the thiocyanogen procedure gives a valuable index as to the types and amounts of the fatty acids present. Some workers have found difficulty in using the method, but if Kaufmann's procedure is followed, Griffiths and Hilditch (1934) and Kimura (1929) have shown that it gives good results.

The analyses of the lipids obtained from the starch by the three methods described, and the calculated distributions of the fatty acids in the total fatty acids of the lipids, are shown in Table IV.

The methanol extract gave a positive test for glycerol, but the amount was not sufficient for a quantitative determination.

It can be seen, from the above results, that the mixtures of fatty acids obtained after saponification of the lipids extracted from starch

by methanol and those obtained after acid hydrolysis of the starch are very similar in composition. Therefore, in a study of the fatty acids of corn starch, those obtained by either method could be employed with equal accuracy. Diastatic hydrolysis does not appear to liberate all of the fatty acids from starch. The unsaturated acids are liberated more easily than the saturated ones.

TABLE IV

ANALYSES OF THE LIPIDS OBTAINED FROM COMMERCIAL CORN STARCH BY THREE METHODS OF PREPARATION

	Extracted with methanol	Liberated by acid hydrolysis	Liberated by diastatic hydrolysis
Methanol extract (% of starch)	1.10	—	—
Lipids (% starch)	0.61	0.64	0.55
Acid value	164.4	195.1	177.0
Saponification value	186.6	—	205.6
Ester value	22.2	—	28.6
Iodine value:			
Rosenmund-Kuhnhenn	88.5	85.9	—
Wijs	93.5	90.5	93.5
Thiocyanogen value	58.2	57.8	65.3
Unsaponifiable matter (% of lipid material)	8.0	5.2	8.0
Phosphatids (% of lipid material)	0.7		0.6
Total fatty acids (% of lipid material)	91.0	93.8	90.5
Acid value	203.0	202.8	202.5
Mean molecular weight	276.4	276.5	277.0
Iodine value:			
Rosenmund-Kuhnhenn	85.9	86.4	—
Wijs	93.2	93.6	103.2
Thiocyanogen value	63.6	64.2	70.7
Saturated fatty acids (Twitchell) (% total f.a.)	30.5	30.0	23.0
Iodine value	0.8	0.9	0.9
Mean molecular weight	268.2	269.0	267.9
Unsaturated fatty acids (by diff.) (% total f.a.)	69.5	70.0	77.0
Mean molecular weight	280.1	280.4	280.8
Iodine value:			
Rosenmund-Kuhnhenn	123.0	122.2	—
Wijs	134.0	133.0	131.6
Oleic acid (% of total fatty acids)	37.0	37.7	41.3
Linolic acid (% of total fatty acids)	31.5	31.1	34.3
Linolenic acid (% of total fatty acids)	1.0	1.2	1.4

The results of the present work show that the major portion of the fatty acids are removed in the free form by both methanol extraction and diastatic hydrolysis. The methanol extraction would not be expected to hydrolyze the fatty acid esters, for, if it did, the acids in the extract would have been in the form of methyl esters since the condition that favors hydrolysis would also favor the formation of methyl esters.

In order to ascertain if the malt extract contained lipases that would split fatty acids from a glyceride, olive oil was subjected to the action of the extract. To 4 ml. of olive oil, 60 ml. of malt extract was added and the mixture was digested for 24 hours at 50°C. At the end of this period the material was cooled and filtered. The filter and residue were dried in a vacuum oven at 50°C. for 8 hours and then extracted with ether for 4 hours. Before this treatment the olive oil had an acid value of 1.84 and after treatment 1.87. This shows that there is very little, if any, hydrolysis of the glycerids by malt extract.

In view of the above it may be concluded that most of the fatty acids, present in starch, are in the form of free fatty acids.

Analysis of the Lipids Removed from Starch at Various Degrees of Extraction with Methanol and the Analysis of the Extracted Starch for Fatty Acids and Phosphorus

1. Lipid analysis.—In the course of the study of methods for extracting lipids from starch, it was found that the iodine value for the extracted fat was highest when the smallest amount of material had been removed. Accordingly, it seemed of interest to analyze the fatty material liberated, and also that remaining in the starch, at different degrees of extraction.

Starch was extracted with methanol in the following manner: A 2,000-g. sample of starch (11% moisture) was suspended in 3 liters of methanol and stirred for 4 hours and then allowed to stand overnight. One liter of the solvent was withdrawn from above the starch cake, filtered, and a portion of the methanol distilled off. A liter of methanol was added to the starch cake and the mixture was stirred for 2 hours. It was allowed to stand for an additional 2 hours, then a liter of solvent was withdrawn, filtered, and distilled. This process was continued until 12 liters of solvent had been removed. The starch was then collected on a Büchner funnel and dried, first in air and then in an oven at 60°C. This procedure removed 57% of the total lipids in the starch.

To obtain a higher removal of fatty material, a 980-g. sample of starch was extracted in a Stokes' extractor for 12 hours. This extracted 81% of the total lipids.

The lipids were obtained from the methanol extracts by adding sufficient ether to make the mixture about 30% ether. This was dried over anhydrous sodium sulfate for 2 days, and after filtering the solvents were distilled off under reduced pressure in a stream of nitrogen, the temperature never exceeding 40°C. This residue was extracted with ether, the solution filtered, and made up to volume. The methods of analysis were the same as those described previously.

Lipids remaining in the extracted starches were obtained by acid hydrolysis of the material followed by collection, washing, drying, and extraction of the flocculent substance formed by hydrolysis. This procedure has also been described. Results of this work are given in Table V.

TABLE V

ANALYSIS OF THE LIPIDS OBTAINED FROM AND REMAINING IN COMMERCIAL CORN STARCH AT VARYING DEGREES OF COMPLETENESS OF EXTRACTION WITH HOT METHANOL

	Lipids removed by methanol extraction			Lipids remaining in starch—liberated by acid hydrolysis		
Total fat extracted	57%	81%	95%	—	—	—
Total fat remaining	—	—	—	19%	43%	100%
Lipids (% of starch)	0.36	0.52	0.61	0.11	0.30	0.64
Acid value	165.1	177.0	164.4		195.6	195.1
Iodine value:						
Rosenmund-Kuhnhenn	105.5	92.7	88.5		49.9	85.9
Wijs	111.2	104.0	93.5		53.0	90.5
Thiocyanogen value	74.7	64.8	58.2		46.2	57.8
Unsaponifiable matter (% of lipid material)	10.0	9.0	8.0	4.0	3.0	5.2
Total fatty acids (% of lipid material)	89.1	90.4	91.0	95.1	96.1	93.8
Acid value	199.5	201.5	203.0	205.8	204.2	202.8
Mean molecular weight	280.7	278.5	276.4	272.8	274.5	276.5
Iodine value:						
Rosenmund-Kuhnhenn	109.7	96.0	85.9	43.5	57.6	86.4
Wijs	115.8	104.5	93.2	47.2	61.4	93.6
Thiocyanogen value	76.4	72.0	63.6	31.0	45.2	64.2
Saturated fatty acids (Twitchell) (% total f.a.)	16.2	21.5	30.5	66.3	50.0	30.0
Iodine value	0.9		0.8	0.8		0.9
Mean molecular weight	267.7	267.5	268.2	269.3	269.0	269.0
Unsaturated fatty acids (by diff.) (% total f.a.)	83.8	78.5	69.5	33.7	50.0	70.0
Mean molecular weight	280.8	281.3	280.1	278.1	282.0	280.4
Iodine value:						
Rosenmund-Kuhnhenn	126.8	122.4	123.0	128.5	115.0	122.2
Wijs	137.7	132.0	134.0	140.0	123.4	133.0
Oleic acid (% of total fatty acids)	40.5	42.8	37.0	15.9	32.2	37.7
Linolic acid (% of total fatty acids)	42.4	34.4	31.5	17.1	17.6	31.1
Linolenic acid (% of total fatty acids)	0.9	1.3	1.0	0.7	0.2	1.2

The results in Table V show that the unsaturated fatty acids are more easily removed by methanol extraction than are the saturated acids. This can be accounted for in the light of the following experiment in which it was found that methanol-extracted corn starch preferentially adsorbed palmitic acid from a methanol solution of palmitic and oleic acids.

A solution containing 10.7 g. oleic acid and 8.7 g. palmitic acid in sufficient methanol to make 100 ml. was divided into two equal

portions. One portion was poured into a flask containing 10 g. of fat-free starch (extracted with methanol) and the other portion was filtered through filter paper. The flask containing fatty acids and starch was shaken for 20 minutes and then filtered. The solids and iodine value were determined on 5-ml. aliquots of the filtrates. It was found that whereas the fatty acid mixture in the control filtrate showed an iodine value of 50.1 and a total solid content of 0.9694 g. in a 5-ml. aliquot ,the fatty acid mixture obtained in the filtrate from the starch showed an iodine value of 59.3 and a total solid content of 0.9420 g. in a 5-ml. aliquot.

As the fatty acids in the filtrate from the starch were more unsaturated than the acids in the control, it can be concluded that more palmitic than oleic acid had been adsorbed by the starch. The saturated acids are held more firmly by starch than are the unsaturated acids and hence are more difficult to remove.

The above data also show that, in a study of the fatty acids removed from a starch by methanol extraction, care must be taken to remove all of the lipids in order to obtain the correct acid composition.

2. *Phosphorus content of extracted starch.*—Taylor (1928) has pointed out an interesting correlation between the phosphorus content of a few common starches and their associated fatty acid content. He observed that the starches which were low in fatty acid were correspondingly high in phosphorus content. It was considered of interest to follow the change in phosphorus content of the starch during the fractional removal of the lipids by methanol extraction. The phosphorus content of the starches was determined by the method of Morris, Nelson, and Palmer (1931).

It was found that, whereas the original starch contained 0.016% phosphorus (dry substance basis), after 57% of the lipids had been removed by methanol extraction, the phosphorus content of the starch was 0.015%. After 81% had been removed, the phosphorus content of the starch was 0.010% and, after 95% had been removed (highest fraction of total lipids removable by methanol), the phosphorus content was still 0.010%. Phospholipids in the methanol extract (95%, see Table IV) amounted to only 0.7% of the total weight of the lipids present. This corresponds to only 0.00016% phosphorus, as phospholipid, in the starch. Methanol extraction removed phosphorus, however, to the extent of 0.006% of the weight of the starch. It is apparent, then, that the major part of the phosphorus removed by the methanol extraction of the starch is not identifiable as phospholipid. No attempt was made to identify this major portion of the extractable phosphorus.

Identification of the Unsaturated Fatty Acids

1. Bromination, separation and identification of the bromides.—A quantity of unsaturated fatty acids (separated by the Twitchell procedure) was dissolved in absolute ethyl ether and brominated according to the procedure of Bailey and Johnson (1918), using bromine dissolved in glacial acetic and a reaction temperature of 2°–3°C. Duplicate samples were run through the bromination and procedure for the separation of the bromides.

After addition of sufficient bromine (so that a deep red color remained in the solution) the mixture was allowed to stand in a refrigerator at about 0°C. for from 16 to 24 hours, then filtered through a sintered glass crucible (10G3) and washed with three 10-ml. portions of ice-cold dry ethyl ether. (The absolute ether was prepared by washing c.p. ether three times with one quarter of its volume of 3% potassium permanganate. It was then washed twice with a saturated solution of calcium chloride and allowed to stand over dry calcium chloride, then filtered and stored over sodium.) The residue that remained on the filter, and which was found to be completely soluble in benzene, is designated, in accordance with the nomenclature of Kaufmann and Keller (1929), as α-hexabromide and corresponds, therefore, to a part of the linolenic acid present in the mixture of unsaturated acid. (The β- or soluble hexabromide is neglected in the subsequent analysis of the bromides.)

The ether was evaporated from the filtrate under diminished pressure, the last portion being removed by heating on a steam bath. The residue was dissolved in a small volume of petroleum ether (b.p. 30°–60°), then refluxed, cooled, and put in a refrigerator at 5°C. for 16 hours. The precipitate was filtered off on a sintered glass crucible, washed with cold petroleum ether, and dried at 95°C. The precipitate is designated as α-tetrabromide and corresponds to part of the linolic acid present in the original mixture of unsaturated acids. The precipitates from the duplicate determinations were combined, dissolved in petroleum ether, decolorized with "Norit," and recrystallized. White needles were obtained which melted from 113° to 115°C. (Dean, 1938, gives 114°C. as the melting point for linolic α-tetrabromide.) Bromine determinations, made on duplicate samples of this precipitate by the method described by Tolman (1909), gave weights of silver bromide that corresponded to an average of 53.6% bromine in the compound. The theoretical value for linolic tetrabromide in 53.33%.

The petroleum ether filtrate from the linolic α-tetrabromide was evaporated to dryness and taken up with dry ethyl ether. The ether solution was washed with sodium sulfite to remove the excess bromine,

and then evaporated to dryness. The bromine content of this residue was determined by the same procedure as was used previously. The silver bromide produced corresponded to an average of 42.05% bromine in the residues from the duplicate separation procedures. The theoretical bromine content of oleic dibromide is 36.18%. The high value obtained is due to the presence of some linolic tetrabromide (the so-called β-tetrabromide).

On the assumption that no hexabromides and that only oleic dibromide and linolic tetrabromide are present in the residue, its relative composition was calculated in the following manner:

$$X = \text{oleic dibromide (\% of residue)}$$
$$100 - X = \text{linolic tetrabromide (\% of residue)}$$
$$36.18X + 53.33(100 - X) = 42.05$$
$$X = 65.8\% \text{ oleic dibromide}$$
$$100 - X = 34.2\% \text{ linolic tetrabromide } (\beta)$$

Results of these separations and calculations are shown in Table VI.

TABLE VI

ANALYSIS OF DUPLICATE SAMPLES OF UNSATURATED FATTY ACIDS BY
BROMINATION AND SEPARATION OF BROMIDES

	Samples	
	1	2
	g.	g.
Mixed unsaturated acids	1.600	1.718
α-Hexabromide (insol. in ethyl ether)	0.036	0.036
α-Tetrabromide (insol. in pet. ether)	0.685	0.827
Residue (soluble in pet. ether)	1.980	2.152
Dibromide (calculated, see text)	1.303	1.420
β-Tetrabromide (calculated, see text)	0.677	0.732

In Table VII the values for the composition of the unsaturated acids as determined by the bromination method are compared with those obtained by application of the Kaufmann and Keller equations as already described.

The remainders of the oleic dibromide and linolic β-tetrabromide mixtures were combined. This was debrominated by the method of Rollet (1909). The fatty acids were recovered by saponifying the esters with 4% potassium hydroxide, followed by acidification. The acids were taken up in petroleum ether, washed, and then dried with anhydrous sodium sulfate. The iodine value (Wijs) of the recovered acid was 110.8, whereas the theoretical iodine value for oleic acid is 89.9.

The p-phenylphenacyl ester of this material was prepared according to Drake and Bronitsky (1930), and after three recrystallizations from alcohol, the ester had a melting point of 59.5°C. These workers

report a melting point of 60.5°C. for the *p*-phenylphenacyl ester of pure oleic acid.

  2. The lithium salt of oleic acid.—Moore (1919) used the lithium salt for the preparation of pure oleic acid.   He pointed out that, when a divalent element such as barium is used for the separation of monovalent acids, there is a possibility of double salt formation, as barium oleolinolate, and for this reason he preferred the use of a monovalent element.   Neither the barium nor the lithium salts effect a quantitative removal of oleic acid from a mixture of unsaturated fatty acids, but both are useful for the qualitative detection of oleic acid.

TABLE VII

A COMPARISON OF THE RELATIVE COMPOSITION OF THE UNSATURATED FATTY ACIDS
PRESENT IN STARCH AS DETERMINED BY THE BROMIDE SEPARATION
PROCEDURE AND BY THE KAUFMANN AND KELLER TECHNIQUE

	By bromation	By K and K equation
Oleic acid (% of total unsat. acids)	52.0⎫52.3 52.6⎭	53.2
Linolic acid (% of total unsat. acids):		
From α-tetrabromide	20.0⎫20.8 21.6⎭	—
Brom β-tetrabromide	19.8⎫19.9 20.0⎭	—
Total	40.7	45.4
Linolenic acid (% of total unsat. acids):		
From α-hexabromide	0.84⎫0.82 0.79⎭	1.4

  For the formation of lithium oleate from the unsaturated fatty acids from corn starch, 2.75 g. of the unsaturated acids, separated by the Twitchell method, were dissolved in 15 ml. of absolute ethyl alcohol and heated to boiling under a reflux condenser.   Then 0.7 g. of lithium hydroxide dissolved in 15 ml. boiling water was added.   (The solution was alkaline to phenolphthalein.)   The solution was cooled slowly and placed in the refrigerator for 12 hours.   The precipitate was filtered off and washed with 50% alcohol.

  The crystalline lithium salt was recrystallized two times from 50% ethyl alcohol and then added to a 10% hydrochloric acid solution to liberate the oleic acid.   The fatty acid was extracted with ether, the ether solution washed with water four times, dried with anhydrous sodium sulfate, and made up to 250 ml.   The ether was evaporated from a 50-ml. aliquot.   The residue weighed 0.0950 g., making 0.475 g. from the sample of 2.75 g. of unsaturated fatty acids.   The yield was 17.3%, which is lower than the percentage (52.3) obtained by difference in the bromination procedure or the value (53.2) by Kaufmann and

Keller calculation method, which shows that a quantitative yield was not obtained.

The iodine number of the acid was 87.7 (Wijs), which indicates a rather pure oleic acid.

This result is another proof of the presence of oleic acid in the fatty acids from corn starch.

3. Identification of the saturated fatty acids.—The saturated fatty acids were separated by the Twitchell method from the total fatty acids liberated from corn starch by acid hydrolysis. From 80.0 g. of total fatty acids, 24.0 g. of saturated fatty acids was obtained which had an iodine value of 0.7 and a mean molecular weight of 269 by titration. The acids were esterified to the methyl esters by the procedure described by Jamieson and Baughman (1920).

TABLE VIII

DATA ON THE SEPARATION OF THE METHYL ESTERS OF THE SATURATED ACIDS OF STARCH LIPIDS AND ON THE IDENTIFICATION OF THESE ACIDS

Methyl esters (14.0 g. total)					Acids recovered		Derivatives— M.P. (°C.)	
Fractions	Boiling range	Weight	% of total	M.P.	Saponification value	Mol. wt.	p-bromphenacyl ester	p-phenylphenacyl ester
	°C.	g.		°C.				
1	145–151	9.66	70.5	30–31	217.7	257.7	85–86	—
2	156–165	3.55	25.9	39	196.7	285.3	90	90.5
3	170	0.49	3.6	55–56	178.8(?)	314.0(?)	89.5	—

Fourteen g. of the mixed methyl esters was distilled at 2 mm. pressure in a micro still equipped with a heated column. Three fractions were obtained, one of which remained in the distillation bulb.

The following boiling ranges were selected because the temperature increased gradually from 145° to 151°C., then moved rapidly to 156°C. From this point to 165° there was another gradual increase and then a rapid increase to 179° where the distillation was stopped. Each fraction was saponified with 4% alcoholic potassium hydroxide, acidified with hydrochloric acid, and the fatty acids obtained in ether. The saponification value was determined for the acid from each fraction and the molecular weight calculated from this.

The *p*-bromphenacyl ester of the acid obtained from each fraction was prepared according to the method of Hann, Reid, and Jamieson (1930) and the *p*-phenylphenacyl ester of the acid obtained in fraction No. 2 was prepared according to the method of Drake and Bronitsky (1930).

The results of these experiments are given in Table VIII. The molecular weights of palmitic, stearic, and arachidic acids are 256.3,

284.3, and 312.3, respectively. Hann, Reid, and Jamieson have reported the melting points of the *p*-bromphenacyl esters of these acids to be 86°C., 90°C., and 89.5°C., respectively. The corresponding derivative of lignoceric acid is given as 90°–91°C. Drake and Bronitsky reported the melting point of the *p*-phenylphenacyl ester of stearic acid to be 90°C.

From these results, the methyl esters of the saturated acids from the starch lipids appear to consist of 70.5% methyl palmitate, 25.9% methyl stearate, and 3.9% of an acid (or acids) of molecular weight higher than that of stearic acid and which appears to be arachidic acid, although the small sample obtained was not sufficient to allow for definite identification.

4. The unsaponifiable fraction.—The percentages of the free and combined sterols in the starch lipids were determined according to the method given by Abderhalden (1925), using digitonin as the precipitating agent. The factor 0.2563 was used to calculate sterol from digitonin steride. This is in accordance with the findings of Sandqvist and Bengtsson (1931), which were confirmed by Windaus, Werder, and Gschaider (1932).

Unsaponifiables amounted to 8.0% of the total lipids (methanol extract). Sterol determination on this unsaponifiable fraction showed it to be 75.8% sterols (6.07% of the total lipids). Free sterols, present in the unhydrolyzed lipids of the methanol extract of the starch, amounted to 1.78% of the total lipids or 29.3% of the total sterols present. The remainder of the sterols were present in combined form in the original lipids.

Summary

Corn starch contains approximately 0.65% of lipids. Starch separated from degerminated corn was found to have the same percentage of "fatty material" as commercially prepared starch; hence the lipids of starch are not present as a result of contact with lipid material during milling. Not a very high percentage of the lipids could be removed from starch by methanol extraction in a Soxhlet extractor (cold solvent) but by using the same solvent and an arrangement as described by Stokes (hot solvent) 93% to 95% of the lipids could be removed. This methanol extract is not made up entirely of fatty acids or lipids. Methanol removed the unsaturated acids more rapidly than the saturated. A portion of the phosphorus of corn starch is removed by methanol extraction. An extraction that removed 95% of the lipids lowered the phosphorus content of the starch from 0.016% to 0.010%. Only a small portion of this phosphorus can be attributed to phosphatides. Small amounts of protein are also removed from starch by methanol extraction.

Lipid samples for analysis were prepared as the ether-soluble material in the methanol extract of the starch, and as the ether-extractable material liberated from the starch by acid and by diastatic hydrolysis. For samples obtained from the methanol extract and the acid hydrolysis, the proportions of fatty acids were the same, while in the case of diastase hydrolysis, it was not possible to liberate all of the fatty acids from the starch. The lipids in the methanol extract are made up largely of free fatty acids. However, a positive test for glycerol was obtained on the lipids obtained by the methanol extraction.

The components of the lipid material (ether soluble) in the extract are: fatty acids, 92.0%, of which 88.0% are free in the starch; unsaponifiable, 8.0%, of which 75% is sterols (30% of total sterols is free); and phosphatides, 0.7%.

The fatty acids liberated from starch by acid hydrolysis were composed of palmitic 21.2%, stearic 7.8%, undetermined saturated acids 1.0%, oleic 37.7%, linolic 31.1%, and linolenic 1.2%. The unsaturated fatty acids were separated and partially identified by the bromination procedure. Oleic acid was also identified by purification through the lithium salt. Acids with unsaturation greater than three double bonds were not found.

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MICROSCOPIC EXAMINATION OF DEVELOPING CORN STARCH [1]

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The microscopic examination of developing starch granules involves problems of a botanical nature, but is of interest to the chemist as it may aid him in his attempts to solve the mysteries of the chemical constitution and the physical and chemical behavior of starch. The present paper reports some observations made on the starch granules from corn kernels at different stages of maturity.

Starch for this study was obtained from a yellow field corn, 403 hybrid, developed by the Agronomy Division at the University of Minnesota, and grown on the University Farm. The shoots were marked with string when the visible silks were three or more in number and two centimeters or less in length. The first ears were picked 15 days after marking. Corn at seven different stages of maturity was harvested: 15, 22, 29, 36, 43, 50, and 57 days after silking.

The kernels were cut from the cob for the 15, 22, and 29-day samples, ground in a meat chopper, and screened through a 60-mesh screen. The material remaining on the screen was washed, the washings being added to the first material that passed through the screen. The combined suspensions were allowed to settle overnight at 4°C., the liquid was siphoned off, and the corn gluten was removed from above the starch cake. The starch was washed two times by resuspending in water and centrifuging in a cup centrifuge. The protein layer above the starch was cut away each time with a spatula. The cake was again suspended in water and then run through No. 17 bolting silk. The bolted suspension was centrifuged, the starch cake being suspended in water and centrifuged three times. The starch was then removed to pans, blotted as dry as possible, broken into small pieces, and air-dried in thin layers.

The 36, 43, 50, and 57-day samples of corn were firm enough to be shelled. It was necessary to soak the 43, 50, and 57-day samples in SO_2 water (0.25% SO_2) at 60°C. for 24 hours in order to be able to process the kernels. After this steeping, the steep water was poured off and the corn was run through a meat chopper, mixed with water and screened, and then carried through the same process as described for the earlier samples.
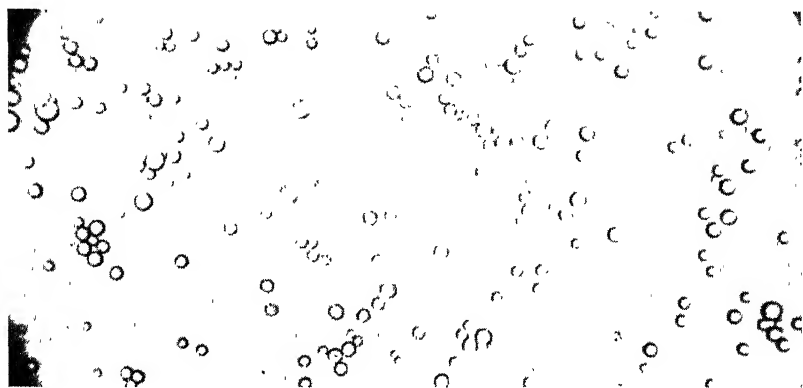
Fig. 1. Photomicrograph of corn starch 15 days after silking (270 ✕).
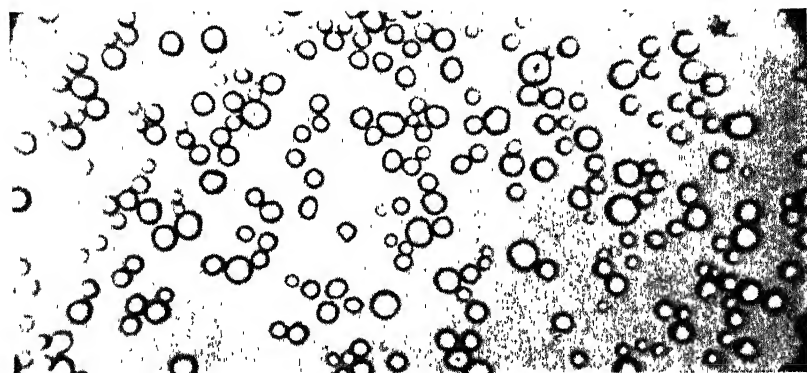
Fig. 2. Photomicrograph of corn starch 22 days after silking (270 ✕).

The starches were suspended in water and photographed (magnification 410 ✕). Photomicrographs of starch prepared from corn at four successive stages of maturity are reproduced in Figures 1, 2, 3 and 4.

The average size of mature corn starch granules is 10 to 25 microns, but deviations in both directions are common. The granules are usually polygonal but many round ones are found.

The photomicrographs of the starch from different aged kernels show a marked difference in size and shape of the starch granule. The outlines of the granules from the 15-day corn (Fig. 1) are round and the granules are much smaller than those from more mature kernels. Starch at this early stage cannot be recognized by microscopic examination as corn starch. At 22 days (Fig. 2) much larger granules
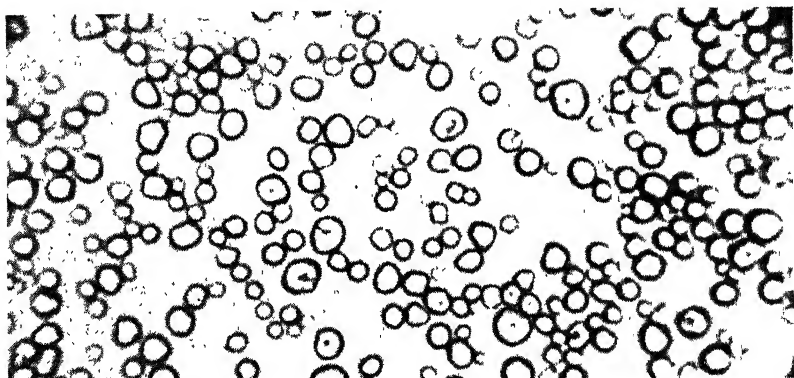
Fig. 3. Photomicrograph of corn starch 29 days after silking (270 ✕).
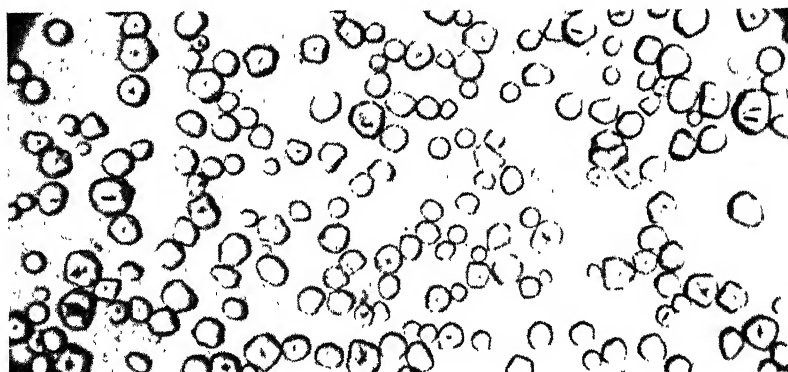
Fig. 4. Photomicrograph of corn starch 36 days after silking (270 ✕).

are present and some of these have polygonal outlines. The starch from 29-day corn (Fig. 3) contains still larger granules, which have increased in relative number in the 36-day sample (Fig. 4). The influence of packing on the shape of the granules can be observed in the photomicrograph of the 36-day sample. The marked granules (-) in Figure 4 (36-day sample) show the indentations made on them by other granules that have been packed around them. There was very little difference between the 36- and 57-day samples.

The starches were observed between crossed nicols and all samples gave a well defined cross.

The photomicrographs presented in this paper show that the starch granules from corn increase in size as the kernel becomes more mature and that their outlines change from circular to polygonal. This change in shape is due to the packing of the granules as the kernel fills and becomes harder.

THE FATTY ACID COMPOSITION OF THE LIPIDS OF CORN STARCH AT VARIOUS STAGES DURING THE DEVELOPMENT OF THE CORN KERNEL [1]

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Several studies have been made of the fatty acids associated with the starch from mature corn kernels (Taylor and Nelson, 1920; Taylor and Lehrman, 1926; Taylor and Werntz, 1927; Taylor and Sherman, 1933; Schoch, 1938). There are no reports in the literature of a study on the fatty acids and other lipids in corn starch prepared from corn kernels at different stages of development. Since information of this type may be of value in leading to an understanding of the formation of starch, the present paper reports the results of such a study.

The starch was separated from corn kernels of various degrees of maturity: namely, 15, 22, 29, 36, 43, 50 and 57 days after silking. The procedure employed has been described in the preceding paper on "Microscopic Examination of Developing Corn Starch" (1941).

Data with regard to the starch yields obtained are given in Table I.

TABLE I

DATA ON STARCH YIELDS OBTAINED FROM CORN KERNELS HARVESTED AT VARIOUS STAGES OF DEVELOPMENT

Days after silking	No. of ears	Weight with husk	Weight without husk	Weight of cut or shelled corn	Starch
		lbs.	lbs.	lbs.	lbs.
15	842	732	234	180.5	3.0
22	227	225	167	80.3	7.1
29	122	113	78	54.0	7.4
36	100	99	80	49.3	10.6
43	69	---	45	36.0	12.0
50	86	- -	58	47.5	16.2
57	132	- -	88	72.1	26.1
Total	1578		750	519.7	82.4

Analysis of the starches for protein, ether extractable substances, lipids obtainable after acid hydrolysis, and phosphorus content are shown in Table II.

Eight hundred- to 1500-gram samples of starch, obtained from corn kernels at each of the various stages of maturity, were hydrolyzed by acid, according to the method of Taylor and Nelson (1920), and the lipids were extracted from the residue with ether. Precautions were taken to prevent oxidation of the lipids by carrying out the hydrolysis and extraction in an atmosphere of nitrogen and drying the extracted material in a vacuum in a stream of nitrogen.

TABLE II

ANALYSIS OF STARCH OBTAINED FROM CORN KERNELS HARVESTED
AT VARIOUS STAGES OF DEVELOPMENT

(Values given as percentages of the starch on dry-weight basis)

	Days after silking						
	15	22	29	36	43	50	57
Protein (N ×6.25)	0.338	0.175	0.263	0.200	0.206	0.200	0.194
Ether extract	0.06	0.05	0.06	0.06	0.05	0.05	0.06
Phosphorus	0.012	0.015	0.018	0.020	0.020	0.019	0.019
Fat "by hydrolysis"	0.49	0.23	0.31	0.49	0.53	0.54	0.54

The lipid material was separated into the saponifiable and unsaponifiable fractions. The fatty acids were liberated from the saponifiable fraction and separated into saturated and unsaturated portions (Twitchell method). The constituent unsaturated fatty acids were calculated by using the iodine (Wijs) and thiocyanogen values of the total fatty acids in Kaufmann's and Keller's equations.

Saturated fatty acids from the 57-day sample were separated by fractional distillation of their methyl esters and were identified by their neutralization equivalents and by their bromphenacyl and p-phenylphenacyl esters.

The methods of analysis were the same as those described in a preceding paper on the "Lipids of Corn Starch" (1941).

. Results of the lipid analyses are given in Table III.

The starch from corn 15 days after silking contained 0.49% "fat by hydrolysis." By 22 days this had decreased to 0.23%, after which it again increased to 0.53% at 43 days, thereafter remaining essentially constant. The decrease in lipid content at 22 days cannot be entirely attributed to dilution due to the rapid increase of starch in the kernel at this period because the proportions of the constituent fatty acids also change. The saturated fatty acids increased from 13.9% of the total fatty acids at 15 days to 27.0% at 22 days, then to approximately 30% at 36 days. From this time to 57 days the relative amount

remained practically constant. A probable explanation for the increase in saturated fatty acids lies in the preferential adsorption of saturated fatty acids by starch.

TABLE III

Analysis of the Lipids from Starch Obtained from Corn Kernels Harvested at Various Stages of Development

(All values are on dry-weight basis)

	Days after silking						
	15	22	29	36	43	50	57
Fat "by hydrolysis" (% of starch)	0.49	0.23	0.31	0.49	0.53	0.54	0.54
Acid value	190.7	177.3	186.1	195.5	196.0	195.4	196.1
Iodine value:							
Rosenmund-Kuhnhenn	117.8	87.8	86.4	83.0	83.3	82.7	83.0
Wijs	127.5	99.9	96.2	88.3	89.3	88.8	89.1
Thiocyanogen value	67.6	55.3	54.2	58.1	57.2	57.9	57.3
Unsaponifiable matter (% fatty material "by hydrolysis")	5.0	13.2	8.0	5.2	5.1	4.7	5.3
Total fatty acids (% fatty material)	94.8	86.4	91.6	94.0	94.9	94.6	94.0
Acid value	201.1	204.2	202.0	203.0	203.1	202.7	202.5
Mean molecular weight	279.0	278.1	277.8	276.2	276.2	276.7	277.0
Iodine value:							
Rosenmund-Kuhnhenn	104.0	89.1	88.0	87.1	86.6	86.0	86.9
Wijs	114.4	97.1	96.2	94.6	93.8	93.6	93.9
Thiocyanogen value	78.1	67.1	65.6	64.2	63.7	63.1	63.6
Saturated fatty acids:							
(Twitchell) (% total f.a.)	13.9	27.0	28.4	30.1	30.8	31.2	30.8
Iodine value	0.8	1.0	1.1	0.7	0.9	1.0	0.8
Mean molecular weight	268.3	268.8	267.8	269.2	269.0	268.2	268.8
Palmitic (% total f.a.)	—	—	—	—	—	—	22.0
Stearic (% total f.a.)	—	—	—	—	—	—	7.8
Undetermined (% total f.a.)	—	—	—	—	—	—	1.0
Unsaturated fatty acids (by diff.) (% total f.a.)	86.1	73.0	71.6	69.9	69.2	68.8	69.2
Mean molecular weight	280.7	281.0	279.9	280.0	280.3	279.2	280.5
Iodine value:							
Rosenmund-Kuhnhenn	123.0	122.4	122.8	123.9	124.4	123.6	123.9
Wijs	132.1	131.8	132.4	133.5	134.1	133.6	134.1
Oleic acid (% total f.a.)	46.2	40.1	38.0	36.5	36.2	35.3	36.0
Linolic acid (% total f.a.)	39.4	31.5	32.5	32.1	31.6	32.3	31.9
Linolenic acid (% total f.a.)	0.5	1.4	1.1	1.3	1.4	1.2	1.3
Unsaponifiable matter (% of fatty material from methanol extract)	—	—	—	—	—	—	7.5
Sterols (% unsaponifiable matter)	—	—	—	—	—	—	75.7
Total sterols (% fatty material)	—	—	—	—	—	—	5.68
Free sterols (% fatty material)	—	—	—	—	—	—	1.84
Combined sterols (% fatty material)	—	—	—	—	—	—	3.84

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CHANGES IN THE BIOCHEMICAL COMPOSITION OF THE CORN KERNEL DURING DEVELOPMENT [1]

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A survey of the literature indicates that relatively few studies have been made concerning the variations in many of the biochemical constituents of the corn kernel, during the developmental stages. Lampe and Meyers (1925) made a study of the carbohydrate storage in the endosperm of sweet corn. Lampe (1931) reported the results of a microchemical and morphological study of the developing endosperm of maize. Zeleny (1935) traced the distribution of nitrogen in the corn kernel at different stages of maturity. The present paper reports the results of analyses of corn kernels at various stages of maturity in which moisture, crude protein, crude fiber, starch, sugars, ash, and ether extract were determined on the samples. Iodine values and free fatty acids were determined on the ether extract.

Preparation of Samples

Samples of a yellow field corn, 403 hybrid, were collected from the fields of the University Farm at St. Paul, Minnesota, at 15, 22, 29, 36, 43, 50, and 57 days after silking. This was accomplished by marking the shoots with string when the silks were 2 cm. or less in length. The lower limit was taken when 3 silks were visible. After the elapse of the desired time, the ears were picked and the kernels were removed from

the cob immediately. A small sample was taken for moisture determinations, and the remainder dried in a vacuum oven at 70°C. for 10 hours and stored in the refrigerator at 5°C. Whole kernels were selected and ground in a mortar to obtain material for the above-mentioned determinations.

Fifteen days after silking, the corn was very green, the kernels were about one-fifth mature size, and the silks were still quite green. In the 22-day sample the kernels were nearly full grown, still very "milky," and the silks were brown. The kernels were starting to dent in the 29-day sample and were only slightly "milky." The husks were just start-

TABLE I

COMPOSITION OF FRESHLY HARVESTED KERNELS

	Days after silking						
	15	22	29	36	43	50	57
Moisture, %	85.4	72.6	60.2	47.6	42.1	40.6	40.0
Dry substance, %	14.6	27.4	39.8	52.4	57.9	59.4	60.0
Crude protein ($N \times 6.25$), %	2.82	3.83	4.76	6.19	6.70	6.90	6.95
Starch, %	3.3	16.0	28.3	37.6	41.4	42.7	43.0
Sugars (reducing) as:							
Dextrose, %	0.77	0.96	0.79	0.42	0.43	0.48	0.44
Sucrose, %	0.56	0.77	0.93	1.26	1.36	1.43	1.40
Crude fiber, %	1.1	1.0	0.9	1.0	1.0	1.0	1.0
Ether extract, %	0.55	1.17	2.03	2.72	3.02	3.08	3.10
Ash, %	0.49	0.74	0.79	1.01	1.05	1.06	1.07

ing to turn brown. By 36 days the kernels were all dented and the husks were browner, and in 50 days the husks were dry and were bursting open.

Methods

Moisture content was determined at the time of harvesting by first drying the samples in a vacuum oven at 70°C. for 4 hours, then grinding the samples, and drying again in a vacuum oven at 100°–105°C. to constant weight. Protein (Kjeldahl-Gunning-Arnold method, using copper sulfate as a catalyst), ash, crude fiber, starch (diastase method with subsequent acid hydrolysis), reducing sugars, and sucrose (acid hydrolysis at room temperature), were all determined by official A.O.A.C. methods (1935).

In order to obtain the ether extract for iodine value and free fatty acid determinations, dried corn was extracted with anhydrous ether *j* Soxhlet extractor. The ethereal solution was dried over anhy sodium sulfate, filtered, and made up to a definite volume with ether. The amount of extracted material was determined by
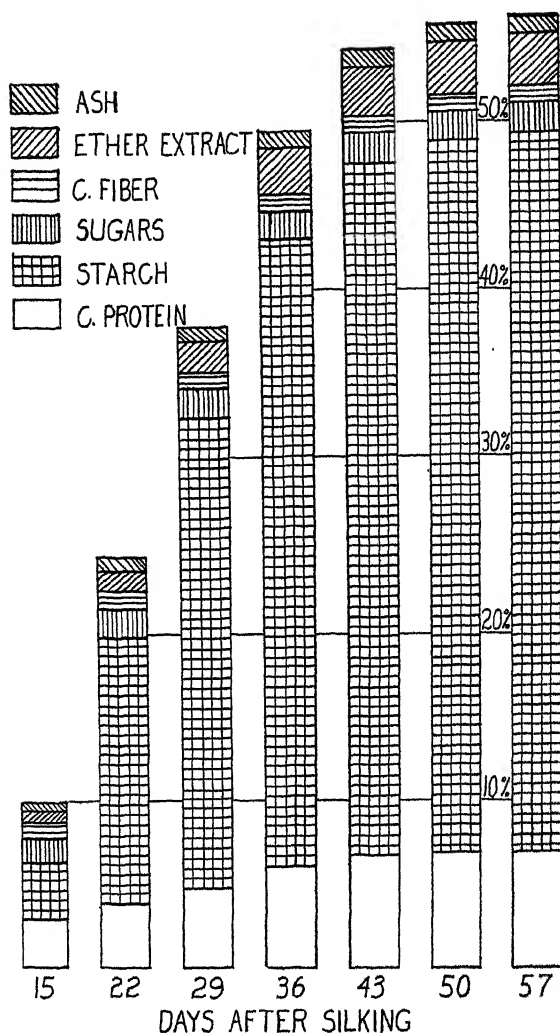
Fig. 1. Composition of corn kernels at various stages of growth, based on corn as harvested

the residue after evaporating the ether from an aliquot. Iodine values (Wijs) and acid numbers (by A.O.A.C. methods) were obtained on aliquots from which the ether had been evaporated under diminished pressure in a stream of nitrogen, the temperature never exceeding 40°C.

Results and Discussion

sults of the analyses, reported on the basis of the corn kernels ed, are shown in Table I. These results are shown graphically

in Figure 1. These data show that there was a steady increase in the amounts of crude protein, starch, sucrose, ether extract, and ash in the corn kernels during their development. There was an increase in the percentage of reducing sugars from 15 to 22 days after silking, then a decrease to a more or less constant value after 36 days. There was little change in the percentage of crude fiber in the kernels.

TABLE II
COMPOSITION OF KERNELS ON DRY MATTER BASIS

	Days after silking						
	15	22	29	36	43	50	57
Crude protein ($N \times 6.25$), %	19.28	13.99	11.96	11.82	11.57	11.61	11.59
Starch, %	22.8	58.3	71.0	71.8	71.5	71.8	71.6
Sugars (reducing) as:							
Dextrose, %	5.24	3.49	1.99	0.80	0.75	0.81	0.74
Sucrose, %	3.80	2.82	2.34	2.40	2.35	2.41	2.33
Crude fiber, %	7.2	3.6	2.2	1.7	1.7	1.7	1.7
Ether extract, %	3.78	4.26	5.11	5.19	5.22	5.18	5.17
Ash, %	3.33	2.71	1.99	1.92	1.82	1.78	1.78
Undetermined, %	34.6	10.8	3.4	4.3	5.1	4.7	5.1

The above results of the analysis of the biochemical composition of corn kernels at various stages of development, calculated to the dry-substance basis, are given in Table II. These results are shown graphically in Figure 2. The percentages of starch and ether extract increased steadily to 36 days, at which time they reached a constant value. Crude

TABLE III
CHARACTERISTICS OF FAT FROM CORN KERNELS AT DIFFERENT STAGES OF MATURITY

	Days after silking						
	15	22	29	36	43	50	57
Ether extract (dry-substance basis), %	3.78	4.26	5.11	5.19	5.22	5.18	5.17
Iodine value (Wijs)	100.8	112.2	123.0	127.7	127.3	126.9	127.1
Acid value	67.2	58.8	54.5	23.5	13.2	13.5	13.4
Free fatty acids as oleic (% ether extract)	33.7	29.6	27.4	11.8	6.7	6.8	6.8

protein, crude fiber, sugars, and ash decreased as the seed matured. These reached a constant value in about 43 days.

Iodine values (Wijs), acid values, and free fatty acids were determined on the ether extracts from the corn samples. The results are shown in Table III. The iodine values (Wijs) of the ether extracts of
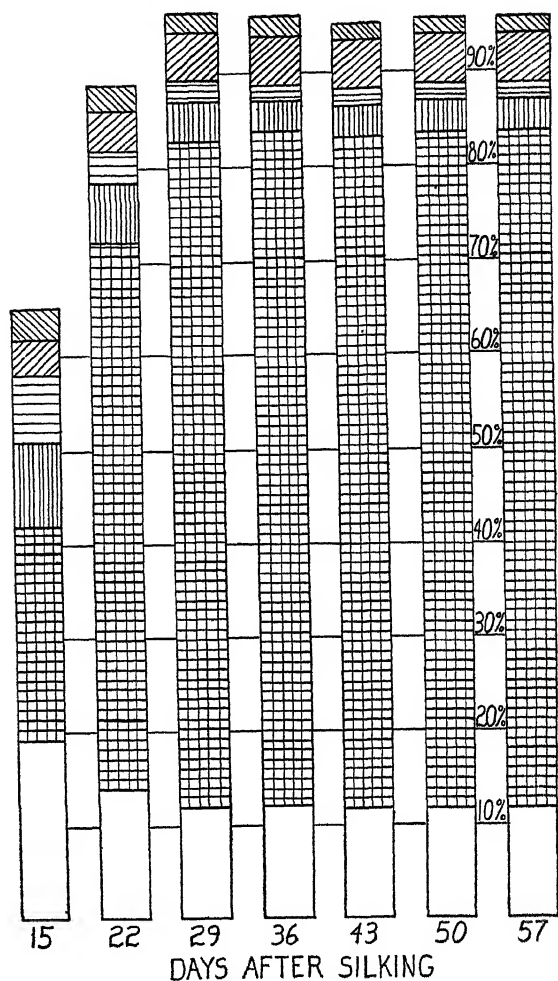
Fig. 2. Composition of corn kernels at various stages of growth, dry-substance basis.
(See Fig. 1 for interpretation.)

corn kernels at successive stages of growth increased from 100.8 at 15 days after silking to 127.7 at 36 days. It appears that oil formation sets in at an early stage and that the nature of the oil progressively changes, becoming more and more unsaturated in character. The percentages of free fatty acids in the ether extracts, calculated as oleic acid, decreased from 33.7% at 15 days to 6.7% at 43 days and remained constant thereafter.

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A MODIFIED THIOCHROME METHOD FOR THE ESTIMATION OF VITAMIN B₁ IN WHEAT AND ITS PRODUCTS

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In the past few months a great deal of attention has been centered on vitamin B_1 in flour and bread. Because of this interest it has been necessary for flour milling laboratories to consider methods for its estimation. Methods of choice should be adaptable for routine analysis and preferably not require unduly expensive equipment. In the literature a number of methods are described, but relatively few satisfy these particular requirements.

There are several biological tests. The rat-growth method is, at present, accepted as standard, but few laboratories have facilities for this type of work. Schopfer's method (1935) is based on the growth of a fungus in the presence of vitamin B_1, but Sinclair (1938) showed it to be not entirely specific. The rate of alcoholic fermentation is powerfully stimulated by vitamin B_1, and Schultz, Atkin, and Frey (1937) used this as a basis for a vitamin assay. They developed a fermentation test and later extended it to a microdetermination (1939). However, it appeared to the present authors that with this method there are many other materials present besides the vitamin, which could conceivably have some action on the fermentation rate. For that reason they have preferred, if possible, to use a method based on some quantitative chemical reaction of the vitamin.

Prebluda and McCollum (1939) first proposed the use of diazotized p-aminoacetophenone which forms a purple-red compound with vitamin B_1, the intensity of the color being a measure of the vitamin concentration. Melnick and Field (1939a, b, c) studied this method,

and extended it to the estimation of phosphorylated thiamin by incubation with yeast phosphatase. To remove materials that might interfere with the reaction, the vitamin is adsorbed on zeolite.

Jansen (1936) pointed out that vitamin B_1 could be measured quantitatively by conversion to thiochrome, a strongly fluorescent material, by oxidation with alkaline ferricyanide. A number of modifications of this test have been devised. Hennessy and Cerecedo (1939) used an enzyme prepared from defatted beef kidney in acid solution for extraction, and subsequent adsorption on Decalso. They measured the fluorescence photoelectrically. Wang and Harris (1939) concluded that the intensity of fluorescence could be estimated equally well by titrating a standard thiochrome solution into a blank and comparing visually. These workers removed pigments and other interfering materials from urine by washing the extracts prior to oxidation with isobutyl alcohol. Pyke (1938) stated that vitamin B_1 was associated in some materials with protein, and for that reason (1939) employed pepsin digestion followed by taka-diastase to liberate any vitamin that might be present in the phosphorylated form. Oxidation was carried out on the unpurified extract and the fluorescence estimated visually.

When considering chemical methods for vitamin B_1 determinations, it becomes apparent that two difficulties are inherent in all of them. Extraction presents a problem due to the fact that the vitamin can exist free and as phosphoric acid esters, and methods must take this into account in order to give a true measure of vitamin potency. Secondly, the listed reactions of the vitamin are influenced by the presence of certain interfering materials. At present little information is available regarding the nature of these interfering compounds, and it is probable that extracts of some materials will contain them while others do not. Hence, the selection of a method should be based on the ease and efficiency with which these problems can be overcome.

Pyke's method appeared the most promising, for the double enzyme treatment seemed to be a logical way to liberate the vitamin. He reported results in good agreement with those of other investigators without resorting to adsorption for freeing the vitamin of interfering materials. The present authors wished to avoid methods employing adsorption because previous experience has shown them to be highly sensitive to manipulation. Furthermore Wang and Harris (1939) and Pyke (1939) reported visual matching of fluorescence with an accuracy comparing favorably with methods involving photoelectric instruments. This is an important item since the latter type of instrument is generally very costly. Thus it was decided to use this procedure for wheat and its products.

Pyke's Thiochrome Method

The thiochrome method of Pyke (1938) is as follows: "20 g. of foodstuff is finely minced or powdered. A solution of 0.1% pepsin in 0.33% hydrochloric acid is added and the volume made up to 97.4 cc. The mixture is incubated overnight at 37°. 2.6 cc. of normal sodium hydroxide and 100 mg. of taka-diastase are added and the incubation continued for another 5 hours. The extracts are then centrifuged. Two 3-cc. aliquots are pipetted into graduated cylinders in which the following reagents are kept stirred by means of a stream of nitrogen. The first cylinder contains 2 cc. of methyl alcohol, 1 cc. of 30% sodium hydroxide, and 1 cc. of 1% potassium ferricyanide. The second cylinder contains 2 cc. of methyl alcohol and 1 cc. of 30% sodium hydroxide only. The nitrogen is kept bubbling for one minute. The solutions are then made up to 10 cc. with water, 13 cc. of isobutyl alcohol is added to each, and they are well mixed. Shaking should not be too vigorous, or troublesome emulsions may be formed. When the supernatant layers become clear, 10-cc. aliquots are pipetted into uniform test tubes, and 1 cc. of methyl alcohol is added to each of them. The tubes are matched by holding them side by side at an angle of 45° against a nickel oxide filter arranged in a vertical position to transmit the ultraviolet light of a mercury vapor lamp. A standard solution of thiochrome is added, 0.1 cc. at a time, to the aliquot prepared without the use of ferricyanide, until the color and intensity of the fluorescence of the two tubes has reached the best possible match."

Preliminary experiments on wheat products proved the following in regard to this method:

1. *Extraction.*—Fineness of grinding is important. The material should be ground as fine as the available equipment permits. In preparing samples for analysis in this laboratory all were ground to pass at least a 50-mesh sieve.

Making up to a total volume of 97.4 cc. presupposes that all materials occupy the same volume. Bran, for example, occupies a considerably larger volume than the same weight of ground wheat, and hence would require less acid-pepsin solution. A definite volume of acid solution should therefore be used.

The greatest activity of pepsin is at pH 2.0 to 2.5; therefore, instead of adding a definite strength of acid, the acidity should be so adjusted that pH of the suspension is in the correct range. This will allow for any differences in buffer value of the material being analyzed.

2. *Presence of interfering materials.*—It was found that the extracts from some materials may be oxidized directly after extraction, but on the whole, interfering compounds must be removed. Particularly is this important with wheat germ; otherwise only 50% to 60% of the

true figure is realized.   According to McFarlane and Chapman (1941) this can be conveniently accomplished by making the solution up to 50% with alcohol and heating to boiling.   This procedure clarifies the extracts and reduces their organic matter content.

However, the use of an alcoholic extract resulted in even greater color in the blank used for the thiochrome titration, and made matching of the two tubes more difficult.   McFarlane and Chapman (1941) found that this could be remedied by the addition of hydrogen peroxide, which largely destroyed the foreign color yet affected no oxidation of the vitamin.   This confirmed the work of Kinnersley and Peters (1928), who failed to oxidize any thiamin to thiochrome with hydrogen peroxide.   The interfering color here mentioned is probably due to the presence of flavone pigments, which are intensely yellow in alkaline solution.

3. Oxidation to thiochrome.—Pyke's method calls for a 3-cc. aliquot of extract to be used for oxidation, but this is not always suitable. When analyzing bread that is relatively low in vitamin compared with other cereal products, the final extract is only feebly fluorescent, whereas a 5-cc. aliquot results in a more easily matched extract. Wheat germ, on the other hand, may contain 1,000 I.U. or more per 100 g., and in that case a 3-cc. aliquot yields an extract that is too bright for convenient matching.   Hills (1939) proved that the relation of concentration to fluorescence is not linear at high concentrations. Therefore, the size of aliquot should be adjusted so that the concentration in the titrated extract is between 0.4 and 3.0 micrograms of vitamin B₁.

It was found that insufficient ferricyanide results in only partial oxidation, whereas an excess destroys thiochrome.   To a great extent, the amount added is dependent on the amount of organic material in solution, for a large part of the ferricyanide is used in oxidation of this material.   Thus the quantity of ferricyanide may not be the same for all classes of materials and should be carefully checked.

It was found also that only partial oxidation may occur if the sodium hydroxide and the potassium ferricyanide are mixed prior to the addition of the extract aliquot.   This was first noticed by a fading of the green color on standing, due no doubt to the reduction of the ferric to the ferrous salt in the presence of strong alkali.   This was overcome by always adding the 30% sodium hydroxide last.

Since this work was completed Booth (1940) has published a paper in which he points out this criticism of Pyke's procedure regarding the order for the addition of the reagents.

The Modified Thiochrome Method [1]

On the basis of findings discussed above, a modified method has been developed, the details of which are presented as follows:

Five grams of finely ground material (through 50-mesh or finer) are weighed into a 125-cc. Erlenmeyer flask, 25 cc. of hydrochloric acid solution containing 25 mg. pepsin is added, and the mixture vigorously shaken. The acid should be of such a strength that the pH of the suspension is approximately 2.0. The sample is incubated overnight at 37°C. The pH of the mixture is then adjusted to 4.7 with approximately normal sodium hydroxide, 25 mg. of taka-diastase is added, and the incubation continued for a further four to five hours. Twenty-five cc. of c.p. methyl alcohol is added and the mixture is heated to boiling under a reflux condenser, cooled, and centrifuged at approximately 2,000 rpm. for 10 minutes. Two aliquots (1 to 5 cc.) are pipetted into 25-cc. glass-stoppered graduated cylinders, containing 2 cc. of methyl alcohol (one for oxidation and one for blank). Three-tenths to 1.0 cc. of fresh 1% potassium ferricyanide is added to the "oxidation" cylinder only, and followed by 1 cc. of 30% sodium hydroxide to both. The cylinders are shaken for approximately one minute, then 1 cc. of 30% hydrogen peroxide is added, they are shaken again, and are then allowed to stand for two to three minutes. The solutions are made up to 10 cc. with water, and 13 cc. of isobutyl alcohol is added and well mixed. The separation of the two layers is accomplished by standing or by centrifuging at medium speed for one minute. Ten cc. of the clear supernatant liquid is pipetted into uniform test tubes containing 1 cc. of methyl alcohol, and these are used for matching.

The pH is best checked after two hours' standing, as the pH of some wheat products will shift appreciably during that time.

The volume of added alkali should be recorded, as the total volume of liquid enters into the final calculation.

No particular advantage is gained by substituting ethyl alcohol for methyl alcohol. However, if it is desired to use denatured alcohol, the result should be checked by means of a sample of known vitamin content.

According to Hills (1939), filtering may be substituted for centrifuging, provided the filter papers have first been subjected to continuous hot extractions with isobutyl alcohol for 24 hours; otherwise some adsorption of vitamin may result.

Glass-stoppered apparatus is preferred throughout, but if corks are used they should be covered with tinfoil to prevent extraction of fluorescent material from them.

[1] Since this manuscript was prepared, it has been found that the dilution of the sample aliquot with 2 cc. of methyl alcohol before the oxidation should be omitted, because this step introduces a serious error with flours of low B_1 content.

It is important that the potassium ferricyanide should be of c.p. quality.

Care should be exercised in the selection of the test tubes; otherwise very serious errors may be introduced. Those used during this study were supplied by the Rubicon Co., for use with the Evelyn Photoelectric Colorimeter. They have an inside diameter of 20 mm. and overall length of 175 mm.

Preparation of Standard Thiochrome

This solution should be prepared fresh daily as it is a relatively unstable compound. The solutions of the vitamin used in the preparation of the standard thiochrome, however, have been found to be stable over a period of months provided they are kept at a pH not higher than 5.0.

It has been found convenient to have two standard thiochrome solutions, the weaker for materials like bread and flour, and the stronger for bran and germ. One-cc. volumes of solutions containing 3.0 or 30.0 μ g. of vitamin B_1 are added to 2 cc. of methyl alcohol in a small separatory funnel. Two drops of 1% potassium ferricyanide are added, followed by 1 cc. of 30% sodium hydroxide, and thorough mixing. Ten cc. of isobutyl alcohol is added, and the mixture vigorously shaken. The aqueous layer is allowed to settle, drawn off, and discarded. One cc. of methyl alcohol is added and the volume made up to 15 cc. with isobutyl alcohol. One cc. of the weaker standard is equal to 0.2 μ g., and the stronger is equal to 2.0 μ g. of vitamin B_1.

The Fluorimeter

A schematic drawing of the apparatus is presented in Figure 1. An 85-watt mercury vapor lamp, with a polished reflector, is mounted approximately 15 cm. behind a nickel oxide filter in a ventilated box. The lamp is set horizontally and parallel to the filter in order to give even illumination for the test tubes directly in front. Many completely assembled units have the lamps at right angles to the filter, but with this arrangement the position of the test tubes is too critical. The test tubes are held side by side in sleeve holders that extend from the top of the tube to within 4 cm. of the bottom and hold them at an angle of 45° to the filter.

Matching is best accomplished by looking down, but at a slight angle so as to compare the fluorescence in the section of the tube from the bottom of the sleeve holders to within 1 cm. of the bottom of the test tubes. This eliminates error due to the variable thickness of the tube bottoms.

The titration of the standard thiochrome from a 10-cc. microburette should be made dropwise with shaking; otherwise errors can be introduced. If this operation is done too quickly, improper mixing results. Under these conditions the blank appears brighter than when
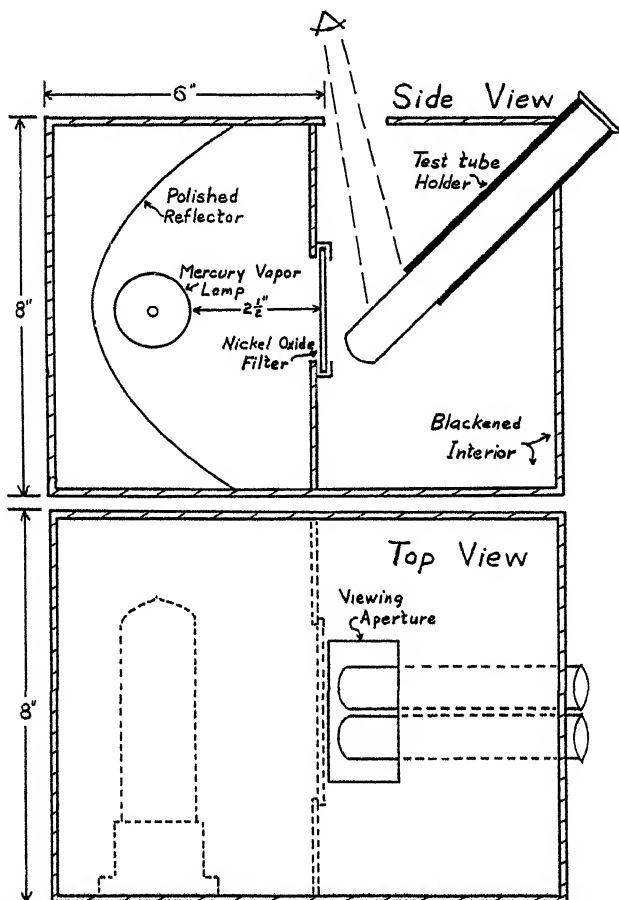
Fig. 1. Visual fluorimeter.

the thiochrome is evenly distributed in the mass of the liquid. The whole operation of matching should be conducted in a darkened room.

In this laboratory it has been the practice to begin an analysis in the late afternoon with the pepsin incubation. The following morning, the pH is readjusted, the taka-diastase is added, and the samples incubated till the early afternoon. Then the oxidations are made and the fluorescence judged. In this way a single operator may conveniently make eight determinations per day.

The following are typical calculations:

$$\text{Aliquot} = 3 \text{ cc.}$$
$$\text{Total vol. liquid} = 55 \text{ cc.}$$
$$\text{Titration} = 1.25 \text{ cc. (weaker standard)}$$

The 3-cc. aliquot represents $3/55 \times 5 = 0.273$ g., original material, but 10 cc. of a total of 17 cc. was used for matching; therefore the 3-cc. aliquot actually represents $10/17 \times 0.273 = 0.161$ g. original material. Vitamin B_1 of sample $= 1.25 \times 0.2/0.161 = 1.56$ μ g. per g. or 52 I.U. per 100 g.

Replicability

As a check on the ability of a person to estimate fluorescence visually, varying amounts of the thiochrome standard were measured into test tubes and made up to 10 cc. with isobutyl alcohol. These were titrated in the usual way. The results in terms of vitamin B_1 are recorded in Table I.

TABLE I

Data from Titration of Known Amounts of Thiochrome
(Expressed as micrograms of vitamin B_1)

Actual concentration	Found	Difference
0.20	0.21	0.01
0.40	0.40	0.00
0.80	0.78	−0.02
1.60	1.66	0.06
3.00	3.02	0.02

. These data confirm the statement of Wang and Harris (1939) that fluorescence can be measured accurately by visual means. However, it should be pointed out that these data do not represent the accuracy attainable with actual cereal extracts because of a faint color in the blank. The hydrogen peroxide treatment does destroy the bulk of the foreign color, but still a trace remains.

Two samples, a flour and a wheat, were analyzed in quadruplicate on successive days. Each extract was also treated in duplicate to indicate the variability within a single determination. These data are presented in Table II.

It appears from Table II that there is as great a variability between aliquots of the same extract as between different extracts. This would indicate that the oxidation process is one of the sources of error. Day-to-day variation also appears to exist, and this is probably due to preparation of the thiochrome standard used for the titration. Therefore particular attention should be paid to these operations. However, it can be concluded that the replicability compares very favorably with vitamin determinations in general.

TABLE II

REPLICATE ANALYSES OF FLOUR AND WHEAT ON SUCCESSIVE DAYS
(Expressed as International Units of vitamin B_1 per 100 g.)

Extract No.	1st day		2nd day	
FLOUR				
1	71	73	73	75
2	73	73	77	75
3	75	75	78	78
4	75	75	77	75
Daily mean		74.4		76.0
WHEAT				
1	136	133	136	140
2	140	136	140	140
3	140	133	140	136
4	140	144	144	136
Daily mean		137		139

Recovery Tests

As a further check on the method, known amounts of thiamin were added to a ground wheat and a flour and the whole procedure followed through in the regular way. The recoveries on the two samples were 97% and 97.5% respectively.

Comparison of Visual and Photoelectric Methods

The Department of Chemistry, Macdonald College, Quebec, kindly consented to collaborate with the authors to determine the variability between two investigators using visual and photoelectric fluorimeters Their instrument has been described by Froman and McFarlane (1940). These data are given in Table III.

TABLE III

ANALYSES BY TWO INVESTIGATORS USING VISUAL AND PHOTOELECTRIC FLUORIMETERS
(Results expressed as International Units of vitamin B_1 per 100 g. material)

	Visual—the authors	Photoelectric—McFarlane and Chapman
Bread	83.5	86
Second clear flour	200	202
Bran	225	215
Wheat germ	925	950

These data indicate good agreement between the two types of measurement, provided the visual matching has been made by an experienced operator. However, it should be pointed out that a certain amount of practice is necessary in order to obtain concordant results, but that is a prerequisite for any type of visual color matching.

Summary

With Pyke's thiochrome method as a basis, a new modified procedure for vitamin B_1 in cereal products has been described and precautions discussed. The method is satisfactory for wheat and its products, and is relatively rapid and easily adaptable for routine work. Because it utilizes a visual method for measuring the intensity of fluorescence, the apparatus required is inexpensive; however, the results compare well with those obtained with the more costly photoelectric instrument. Data on the replicability of the method and recovery of added thiamin have been presented.

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SODA REQUIREMENTS OF CRACKER FLOURS

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In the cracker and biscuit industry the term "soda" is applied to sodium acid carbonate ($NaHCO_3$), and in this work whenever this term is used it also refers to sodium acid carbonate. The various commercial brands on the market are almost chemically pure (99.6% to 99.9% purity). To cracker bakers soda is known as a "chemical," but in the legend on the package soda is usually classified as a "leavener."

The Function of Soda in Fermentation and Baking

The terms "crackers," "soda crackers," and "oyster crackers" (also known as "sponge goods") include the products of various shapes and sizes such as: square (2-inch standard size and 2½-inch larger size), oblong (flake), round (wafer), and small round (oyster).

Crackers are made from fermented doughs of 18 to 23 hours' sponge and 3 to 5 hours' dough fermentation time with 0.2% to 0.5% yeast, 1.0% to 1.25% salt (usually 1.0%), 0.5% to 0.7% soda, 5.0% to 15.0% shortening, and 27% to 35% water (based on the flour as 100). In cookie and biscuit doughs soda combined with an acid base is chiefly used as a leavener, but this is not the case in crackers where soda is used alone with no acid base added. The primary function of soda in crackers is chemical and physico-chemical, rather than to provide a leavening action. As a result of the neutralization of the natural acidity present in the flour as well as the acidity created in the fermentation (in both sponge and dough), the desirable qualities of spring, tenderness, texture, grain, color, and bloom, are secured in the finished product.

The leavening action in cracker fermentation is largely due to the yeast, and on the other hand soda added after 18 to 23 hours of sponge fermentation has a great deal of retarding effect on fermentation and gas production in the dough stage. However, this retarding effect is due to the "free" soda which was not neutralized by the acidity created in fermentation and the natural acidity present in the flour; consequently the larger the amount of the "free" soda the greater is the retarding effect.

The retarding effect of soda on fermentation can be best seen if soda is omitted, when fermentation proceeds at a much faster rate than under the normal conditions, while if the normal amount of

soda is doubled, the fermentation is almost stopped with practically no gas production. Both these extreme cases may occur accidentally in commercial practice. The gas production due to the direct reaction of soda with the acids created in fermentation appears to be negligible as to its contribution to leavening of the finished cracker and is not considered at all in the commercial practice.

There is a certain school of thought who maintain that the main value of soda in baking is due to the leavening action according to the reaction whereby 2 $NaHCO_3$ is decomposed into Na_2CO_3, CO_2, and H_2O. However, this reaction does not occur because there is, and usually there should be, very little or none of the "free" soda (not neutralized) either in the well fermented doughs or in the finished product, if the pH is close to the neutral point or slightly on the alkaline side. $NaHCO_3$ decomposes at 270°C. (518°F.) (*Handbook of Chemistry and Physics*, 1937–38), whereas the baking temperatures of crackers are 232° to 288°C. (450° to 550°F.) with a very short baking time of three to four minutes. Furthermore, the inside temperature of the cracker in the oven during baking is slightly above the boiling point of water 104°C. (219°F.). Finally, the analysis of the baked cracker even with the pH above 8.1 did not show the presence of CO_3'' (Na_2CO_3).[1]

There is a rise in pH during baking, which Johnson and Bailey (1924) attributed to the conversion of the acid carbonate into the normal carbonate "and the loss of the acid reacting carbon dioxide from the dough during the baking process." The same authors also state that "These observations suggested that some reaction other than the bicarbonate to carbonate conversion was involved in effecting changes in hydrogen ion concentration during the baking process." Our tests have shown that there is a similar rise in pH in the acid cracker (for example pH 5.8 of the unbaked cracker was changed to 6.6 after baking), which hardly can be attributed to the conversion. On the other hand we agree on the points that "the loss of the acid-reacting carbon dioxide from the dough during the baking process" is one of the factors responsible for the increase in pH during baking and also that there are still other changes, probably very complex, which will require further study.

Selection and Blending of Cracker Flours

We have for many years made comparisons for quality of crackers in the different plants operated in various sections of the United States. The crackers compared were of good grade (standard, the

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[1] The test for CO_3'' with phenolphthalein was negative, while methyl orange showed the presence of HCO_3' (*Technical Methods of Analysis*, by R. C. Griffin, 1927, pp. 30–31).

TABLE I
ANALYTICAL AND PRODUCTION DATA ON FLOURS USED IN VARIOUS PLANTS

Plant	Dough	Flour brand[1]	Ash[2]	Protein[2]	Viscosity[2,3]	Relative percentage of flours	Relative quantity of protein	Ratio of sponge to dough flours	Relative quantity of protein in sponge, dough, and total flours	Soda per one-barrel batch	Percentage of soda based on flour	pH of the cracker
			%	%		%	%	%	%	lbs.	%	
A	Sponge	1	0.41	9.50	76	20.00	1.90	60.00	5.54			
		2	0.42	9.10	60	40.00	3.64					
	Dough	2	0.42	9.10	60	40.00	3.64	40.00	3.64 / 9.18	1.17	0.60	7.5
B	Sponge	3	0.40	9.50	74	30.00	2.85	60.00	5.76			
		4	0.41	9.70	80	30.00	2.91					
	Dough	3	0.40	9.50	74	20.00	1.90	40.00	3.44 / 9.20	1.22	0.62	7.4
		5	0.38	7.70	24	20.00	1.54					
C	Sponge	6	0.41	9.50	68	14.29	1.36	57.14	5.56			
		7	0.41	9.80	86	42.85	4.20					
	Dough	8	0.40	8.10	33	14.29	1.16	42.86	3.73 / 9.29	1.05	0.54	7.8
		9	0.43	9.00	58	28.57	2.57					
D	Sponge	4	0.41	9.70	80	32.00	2.75	64.00	5.85	.		
		10	0.39	8.60	58	32.00	3.10					
	Dough	11	0.41	8.10	34	36.00	2.92	36.00	2.92 / 8.77	1.09	0.56	7.6

TABLE I—*Continued*

Plant	Dough	Flour brand[1]	Ash[2]	Protein[2]	Viscosity[2,3]	Relative percentage of flours	Relative quantity of protein	Ratio of sponge to dough flours	Relative quantity of protein in sponge, dough, and total flours	Soda per one-barrel batch	Percentage of soda based on flour	pH of the cracker
			%	%		%	%	%	%	lbs.	%	
E	Sponge	12	0.39	8.70	52	14.28	1.24	71.42	6.27	1.30	0.66	7.8
		13	0.42	8.90	62	28.57	2.54					
		14	0.41	8.70	60	28.57	2.49					
	Dough	15	0.54	8.90	26	14.29	1.27	28.58	2.51			
		16	0.39	8.70	60	14.29	1.24		8.78			
F	Sponge	17	0.45	8.50	40	11.91	1.01	71.44	6.25	1.07	0.55	7.6
		18	0.41	8.80	65	35.72	3.14					
		19	0.41	8.80	56	23.81	2.10					
	Dough	17	0.45	8.50	40	10.20	1.47	28.56	2.34			
		20	0.45	8.00	32	18.36	0.87		8.59			
G	Sponge	21	0.38	9.70	77	30.00	2.91	60.00	5.88	1.00	0.51	7.4
		22	0.40	9.90	80	30.00	2.97					
	Dough	23	0.42	8.80	56	40.00	3.52	40.00	3.52			
									9.40			
H	Sponge	21	0.38	9.70	77	30.00	2.91	60.00	5.79	1.35	0.69	7.4
		24	0.41	9.60	74	30.00	2.88					
	Dough	25	0.42	8.90	58	40.00	3.66	40.00	3.66			
									9.45			

TABLE I—Continued

Plant	Dough	Flour brand[1]	Ash[2] %	Protein[2] %	Viscosity[2][3]	Relative percentage of flours %	Relative quantity of protein %	Ratio of sponge to dough flours %	Relative quantity of protein in sponge, dough, and total flours %	Soda per one-barrel batch lbs.	Percentage of soda based on flour %	pH of the cracker
I	Sponge	26	0.42	9.80	56	13.33	1.31	60.01	5.50			
		27	0.40	8.50	35	20.00	1.70					
		28	0.43	9.70	60	13.34	1.29					
		29	0.42	9.00	48	13.34	1.20					
	Dough	30	0.40	8.50	32	13.33	1.13	39.99	3.74	1.03	0.53	7.8
		31	0.41	9.80	58	26.66	2.61		9.24			
J	Sponge	32	0.42	9.80	80	28.57	2.80	71.43	7.04			
		33	0.49	9.90	58	42.86	4.24					
	Dough	34	0.47	9.50	70	28.57	2.71	28.57	2.71	1.00	0.51	7.4
									9.75			
K	Sponge	35	0.42	8.80	56	23.81	2.10	71.43	5.89			
		36	0.40	7.80	36	23.81	1.86					
		37	0.41	8.10	33	23.81	1.93					
	Dough	38	0.42	8.70	58	16.67	1.45	28.57	2.43	1.02	0.52	7.8
		39	0.41	8.20	26	11.90	0.98		8.32			

[1] Flour brands are recorded in code by number rather than by names.
[2] Based on 13.50% moisture.
[3] Based on 5 cc. normal lactic acid reading by the MacMichael viscosimeter.

TABLE II

Unusual Cases of Flour Blends (Patents, Cut-Straights and Clears), Blends with Spring Wheat Flours, and Laboratory-Made Crackers

Batch	Dough	Flour brand[1]	Ash[a]	Protein[a]	Viscosity[a,3]	Relative percentage of flours	Relative quantity of protein	Ratio of sponge to dough flours	Relative quantity of protein in sponge, dough, and total flours	Soda per one-barrel batch	Percentage of soda based on flour	pH of the cracker	Remarks
			%	%		%	%	%	%	lbs.	%		
1	Sponge	40	0.40	8.80	58	50.00	4.40	50.00	4.40				The same brand of flour in the sponge and dough; ratio 50/50.
	Dough	40	0.40	8.80	58	50.00	4.40	50.00	4.40 / 8.80	0.96	0.49	7.4	
2	Sponge	40	0.40	8.80	58	70.00	6.16	70.00	6.16				The same brand of flour as in batch 1 but the ratio is 70/30.
	Dough	40	0.40	8.80	58	30.00	2.64	30.00	2.64 / 8.80	1.20	0.61	7.4	
3	Sponge	41	0.42	9.80	80	50.00	4.90	50.00	4.90				One brand of flour in the sponge and another brand in the dough; ratio 50/50.
	Dough	42	0.40	8.10	42	50.00	4.05	50.00	4.05 / 8.95	1.00	0.51	7.8	
4	Sponge	43	0.39	9.60	75	50.00	4.80	50.00	4.80				The same brand of flour in the sponge and dough blended with another brand in the dough; ratio 50/50.
	Dough	43	0.39	9.60	75	25.00	2.40	50.00	4.40	0.98	0.50	7.6	
		44	0.40	8.00	38	25.00	2.00		9.20				
5	Sponge	45	0.43	9.70	76	60.00	5.82	60.00	5.82				Flours very active in fermentation.
	Dough	46	0.42	8.90	58	20.00	1.78	40.00	3.42	1.50	0.77	7.5	
		47	0.42	8.20	42	20.00	1.64		9.24				

TABLE II—*Continued*

Batch	Dough	Flour brand[1]	Ash[2] %	Protein[2] %	Viscosity[2,3]	Relative percentage of flours %	Relative quantity of protein %	Ratio of sponge to dough flours %	Relative quantity of protein in sponge, dough, and total flours %	Soda per one-barrel batch lbs.	Percentage of soda based on flour %	pH of the cracker	Remarks
6	Sponge	48 49	0.44 0.39	9.40 8.80	65 58	30.00 30.00	2.82 2.64	60.00	5.46				Dough flour too soft.
	Dough	50	0.37	6.70	18	40.00	2.68	40.00	2.68 8.14	0.96	0.49	7.4	
7	Sponge	51 52	0.40 0.38	8.20 7.80	51 29	11.90 35.71	0.98 2.79	47.61	3.77				Flour blend too soft.
	Dough	53 54	0.40 0.15	8.10 7.60	52 17	28.58 23.81	2.31 1.81	52.39	4.12 7.89	0.96	0.49	7.8	
8	Sponge	55 56	0.42 0.40	8.40 8.20	54 48	30.00 30.00	2.52 2.46	60.00	4.98				Flour blend too soft.
	Dough	57	0.44	7.00	40	40.00	2.80	40.00	2.80 7.78	0.91	0.46	7.6	
9	Sponge	58 59	0.42 0.48	9.80 9.90	67 60	28.57 42.86	2.80 4.24	71.43	7.04				Flour blend too strong.
	Dough	60	0.50	9.50	60	28.57	2.71	28.57	2.71 9.75	0.91	0.46	7.7	
10	Sponge	61 62	0.43 0.44	10.60 10.10	92 88	30.00 30.00	3.18 3.03	60.00	6.21				Flour blend too strong.
	Dough	63	0.42	9.70	82	40.00	3.88	40.00	3.88 10.09	0.89	0.45	7.5	

TABLE II—*Continued*

Batch	Dough	Flour brand[1]	Ash[2]	Protein[2]	Viscosity[2,3]	Relative percentage of flours	Relative quantity of protein	Ratio of sponge to dough flours	Relative quantity of protein in sponge, dough, and total flours	Soda per one-barrel batch	Percentage of soda based on flour	pH of the cracker	Remarks
			%	%		%	%	%	%	lbs.	%		
11	Sponge	64	0.42	9.50	72	30.00	2.85	60.00	6.18	1.60	0.82	7.5	Blend of clear and patent flours.
		65	0.62	11.10	26	30.00	3.33						
	Dough	66	0.41	8.10	35	40.00	3.24	40.00	3.24 / 9.42				
12	Sponge	67	0.65	11.60	36	60.00	6.96	60.00	6.96	1.75	0.89	7.5	Clear in the sponge and patent in the dough.
	Dough	68	0.42	8.20	26	40.00	3.28	40.00	3.28 / 10.24				
13	Sponge	69	0.60	10.80	38	60.00	6.48	60.00	6.48	1.70	0.87	7.8	Clear in the sponge and cut-straight in the dough.
	Dough	70	0.52	9.40	45	40.00	3.76	40.00	3.76 / 10.24				
14	Sponge	67	0.64	11.70	38	60.00	7.02	60.00	7.02	1.90	0.97	7.8	Clear in the sponge and dough; ratio 60/40.
	Dough	71	0.75	9.80	16	40.00	3.92	40.00	3.92 / 10.94				
15	Sponge	67	0.65	11.70	36	70.00	8.19	70.00	8.19	1.96	1.00	7.7	Clear in the sponge and dough; ratio 70/30.
	Dough	72	0.60	11.20	38	30.00	3.47	30.00	3.47 / 11.66				

TABLE II—*Continued*

Batch	Dough	Flour brand[1]	Ash[2]	Protein[2]	Viscosity[2,3]	Relative percentage of flours	Relative quantity of protein	Ratio of sponge to dough flours	Relative quantity of protein in sponge, dough, and total flours	Soda per one-barrel batch	Percentage of soda based on flour	pH of the cracker	Remarks
			%	%		%	%	%	%	lbs.	%		
16	Sponge	67 / 73	0.65 / 0.61	11.70 / 11.30	36 / 38	35.00 / 35.00	4.10 / 3.96	70.00	8.06				Two clears in the sponge and one in the dough.
	Dough	74	0.72	9.70	18	30.00	2.91	30.00	2.91 / 10.97	1.88	0.96	7.4	
17	Sponge	75 / 76	0.42 / 0.40	9.60 / 9.50	72 / 70	30.00 / 30.00	2.88 / 2.85	60.00	5.73				Regular commercial batch for standard 2-inch-size cracker.
	Dough	77	0.40	8.40	30	40.00	3.36	40.00	3.36 / 9.09	1.25	0.64	7.6	
18 LABORATORY	Sponge	75 / 76	0.42 / 0.40	9.60 / 9.50	72 / 70	30.00 / 30.00	2.88 / 2.85	60.00	5.73				Same brands of flours and ratio as in batch 17.
	Dough	77	0.40	8.40	30	40.00	3.36	40.00	3.36 / 9.09	—	0.36	7.6	
	Blend with 40% spring wheat Flour brands unknown									0.75	0.38	7.8	Not all production data known.

[1] Flour brands are recorded in code by number rather than by names.
[2] Based on 13.50% moisture.
[3] Based on 5 cc. normal lactic acid reading by the MacMichael viscosimeter.

approximate two-inch square size) and the flours used were mostly 90% to 100% patents, although a few cut-straights were used in some cases.

It can be seen from Tables I and II that the flours used varied widely in protein content from as low as less than 7.0% (Table II; batch No. 6) to as high as more than 10.0% (Table II, batch No. 10). The flours were supplied by mills located as far east as New York and Pennsylvania and as far west as Washington and Oregon; however, the major portion was from the midwest (Kansas, Missouri, Indiana, Michigan, Ohio, and Illinois).

It was observed in some plants that the operators were guided by certain rules in selecting and blending the sponge and dough flours. Some favor sponges consisting entirely of strong flours of 9.5% to 10.0% protein (Table I, B, C, J), while others a combination of strong flours of 9.5% to 10.0% protein with softer-type flours of 8.5% to 9.5% protein (Table I, A, D), whereas still others prefer all soft flours of 8.0% to 9.0% protein (Table I, E, F). In the dough some use soft flours (cookie type, Table I, D, F) while others prefer strong flours (sponge type, Table I, A, J) and still others a combination of strong and soft flours (Table I, B, C). In some cases no distinction was made between sponge and dough flours, and accordingly either all strong or all soft flours for the whole batch (Table I, J, K) were used.

Some operators follow the policy of using the same brands of flours all year, and year after year for the purpose of maintaining a uniform product, while others change brands but retain at least one of the sponge flours. With the same thought in mind some operators limit all flours to three or four brands (Table I, B, C), some to as many as five to six (Table I, E, I), and in some cases one to two, keeping the same brand of flour in the sponge and dough (Table I, A; Table II, batches 1 and 2).

Protein

Because more than two flours of various strengths are usually used per batch (a commercial batch consists of about five to six barrels of flour) and also because of the great variation in the ratio of sponge to dough flours, it was found that more attention should be paid to the quantity of protein contributed by each component flour as reported in Tables I and II under "relative quantity of protein."

The relative quantity of protein is derived by reducing the protein content as given by the analysis to the relative percentage of flour; for example: if the protein is 9.5%, and 25% of the flour was used in the batch, then the relative quantity of protein is one-fourth or 2.38%.

Results that are obtained by adding the figures for the sponge, dough, and total flours as reported in Tables I and II under "Relative quantity of protein of the sponge, dough and total flours" prove very valuable for the selection and blending of the cracker flours.

By comparing the flours in each individual plant and then comparing the flours used between the various plants after a period of several years, it was found that the best results could be obtained if the relative quantity of protein contributed by the sponge flours has a range of 5.5% to 6.5% with an average of 6.0% and for the total flours (sponge and dough) 8.5% to 9.5% with an average of 9.0% (Table I, A to I), bearing in mind that all these flours possessed good fermentation and protein qualities. The crackers—tested over a period of years—made with flours of this range are more uniform in quality, and the doughs apparently have good tolerance because they are less susceptible to unfavorable changes in mixing, fermentation, machining, and baking as a result of changes in the new wheat crop flours. However this does not mean that good crackers are produced only from the flours belonging to this protein range, as there are so many other factors in fermentation and baking that may be operative. On the other hand, good crackers can be produced from the flours exceeding this protein range but the cracker doughs made with these flours do not possess good tolerance and they lack uniformity.

It is to be expected that for flours that are fermented properly, the higher the protein content, the more soda is taken up. This is usually true up to a point where the flours are so strong (Table I, J) that this condition becomes reversed, the fermentation is retarded with less acidity being developed, and consequently a lower amount of soda is used as in the case of soft flour (Table I, K).

In testing crackers over a period of years it was observed that crackers made with either too soft or too strong flours (Table II, batches 7 to 10) were scored low because they lacked in flavor which was described as being "flat," "raw," or "floury." With too strong flours (Table II, batches 9 and 10) the cracker bakers experienced difficulties in manufacturing which caused "buckling" in baking, especially if too stiff doughs were used. "Buckling" is well known among cracker bakers because strong cracker flours are more abundant some seasons than the soft flours. On the other hand, with too soft flours the cracker bakers also experienced difficulties in manufacturing which were manifested by "spreading" or "creeping" of the crackers in baking, especially if too soft doughs were used. The term "creeping" is used when the crackers spread so much on the oven shelf that the sheets touch one another.

Ratio of Sponge to Dough Flours

The relative quantity of protein of the sponge flours is also dependent upon the ratio of the sponge to dough flours. If the amount of sponge and dough flour is the same (ratio 50/50) even with the high protein sponge flours (Table II, batches 3 and 4) the relative quantity of protein for the sponge flours falls below 5.0% and with the soft flours even below 4.5% (Table II, batch 1). Such flours take up less soda and consequently the crackers lack in fermentation flavor (are "raw" and "floury" in eating quality). This is especially true in the case of batch No. 7, Table II, where all flours used were very soft and the ratio was even below 50% (47.61%). The best results are obtained if the major part of the flours (60% to 70%) is used in the sponge.

In blending of cracker flours the point of ratio of sponge to dough flours is sometimes overlooked and for that reason variations in quality of crackers occur from year to year.

Soda

It was found by observation for many years that in the well fermented doughs the flours should take up 0.6% soda (based on the flour as 100) with a range of 0.5% to 0.7%. 0.6% corresponds to 1.176 lbs. soda per one-barrel batch, with a range of 0.98 to 1.372 lbs. (Table I, A to K). In a five-barrel batch (a commercial size) there is a range of 4.9 to 6.860 lbs. with an average of 5.88 lbs. If soda is not adjusted the finished cracker would show a wide variation of pH, as low as 5.0 and as high as 9.0. It must be borne in mind that once soda is adjusted all other variables affecting cracker fermentation such as sponge and dough setting temperatures, fermentation time, sponge room temperature, etc., must be kept constant in order that all finished crackers can be brought to the same pH. In our work the pH of the finished crackers was 7.4 to 7.8, in most cases close to 7.6, showing no appreciable effect of the variation of the soda. However if all other variables affecting the cracker fermentation are kept constant, then any variation in soda will be reflected directly in pH.

In unfermented doughs such as hard-tack and some cookie doughs, the flours take up 0.25% to 0.35% of soda, with an average of 0.30%. Usually 0.75 pound of soda per one-barrel batch of hard-tack is sufficient to bring the pH to an alkaline condition regardless of the grade of flour used. In the fermented doughs (for better-grade crackers) nearly double the amount of soda is used to bring the pH to the alkaline side. This indicates that half of the soda is taken up by the natural acidity present in flour, while the other half is taken up by the acidity created in fermentation. Any variation of soda in the fermented

doughs is largely due to the acidity created in fermentation rather than to variation in the initial (natural) acidity because the flours used are mostly unbleached with the pH 6.0 or close to it.

If less than 0.5% soda is used, because of too low or too high protein in the flours, or as a result of improper ratio of sponge to dough, the crackers lack in fermentation flavor. It is generally believed among cracker bakers that the more soda is taken up by the flours, the more tender and better will be the eating quality of the crackers produced, provided good-grade patent flours are used. This is only true if all of the soda added is taken up by the acidity (natural and created in fermentation), because if there is more soda used, but some of it is present as "free" soda, then this excess of soda causes toughening and lack of spring in the finished product, and if the extreme is reached with the pH exceeding 8.1 (by electrometric determination) then there is an off-flavor and very often "soapy" flavor. It is also a known fact that higher alkalinity affects color of crackers, causing quick browning during baking. Very rarely do patent flours of 90% to 100% extraction take up more than 0.7% soda, but flours may occasionally be encountered that are unusually active in fermentation under most favorable plant and climatic conditions (Table II, batch 5), or if buffer salts are added. Usually if more than 0.7% of soda is added, cut-straights and clear flours (Table II, batches 11 to 16) are used, giving the crackers a strong flavor. The clear flours may take up as much as 1.0% of soda (Table II, batch 15), that is about two pounds per one-barrel batch (1.96 lbs. to be exact), due to a greater fermentation rate resulting in the production of more acid. There is a probability that some acidity in fermentation requiring an extra amount of soda is developed due to bacterial activity, especially if lower-grade clear flours are used and if the yeast is as low as 0.1% and the salt below 1.0% (based on the flour as 100).

The same or similar brands of flours may take up as low as 0.5% of soda under one plant's conditions and as high as 0.7% in another plant (Table I, G, H). This means that the soda requirements of flours, sponges, and doughs are largely governed by the plant and climatic conditions. There may be a correlation of behavior between the same or similar brands of flours at different plants; for instance, if a certain flour takes up an increase of soda of from 0.60% to 0.62% at one plant then a similar increase from 0.65% to 0.67% may occur at another plant. The soda requirements of flour are slightly affected from year to year by a change to new-crop flour.

The fact that soda is a good criterion of cracker fermentation can be observed in the case of newly purchased steel fermentation troughs, for when these troughs are first used the fermentation is retarded to

such an extent that the soda requirements of flours may fall as low as 0.4%, which is just about one-third of the normal fermentation, bearing in mind that the soda requirement of unfermented flour is 0.3% and fully fermented flour 0.6%. The soda should be gradually increased, for it may take as long as two weeks before the same amount of soda is reached as was used in the old troughs.

Similar cases are observed in crackers made from blends of spring and soft winter wheat flours, where the soda may fall as low as 0.38%, showing that practically none of the normal cracker fermentation occurred and that the finished product had all of the characteristics of hard-tack (a product made from an unfermented dough). The spring wheat flours not only do not respond to the usual cracker fermentation but also prevent the normal processes of fermentation in the soft winter wheat flours. Practically the same results were obtained in the experimentally made crackers (in the laboratory), showing that the finished product had all the characteristics of hard-tack, particularly lacking in spring, tenderness and flavor, although the flours used (soft winter wheat) and the amount of shortening were the same as in the crackers manufactured in the plant. The amount of soda was almost as low as in hard-tack (Table II, batches 17 and 18).

It is a well known fact that soda cracker fermentation is a very complex problem and the study of soda requirements of flours is only one factor affecting it. There are many other factors that should be studied alone and in combination to determine the fundamental laws underlying the soda cracker fermentation.

Summary

In crackers the primary function of soda is not a leavening action, but rather it is the neutralization of the initial (natural) acidity present in the flour as well as of the acidity created in fermentation.

Soda is not changed chemically in baking.

The soda requirements of flours in unfermented doughs are 0.25% to 0.35%; in fermented doughs (crackers) made with patent flours, 0.5% to 0.7%; with cut-straights and clears the requirements may be as high as 1.0%.

The amount of soda is one of the most important criteria of cracker fermentation, provided the crackers are brought approximately to the same pH (7.6).

Soda requirements of flours are affected slightly in changing from old to new crop flours.

Plant and climatic conditions exert a large effect upon the soda requirements of flours, cracker sponges, and doughs.

Too high and too low protein in flours and flour blends with improper ratio of sponge to dough require less soda. This causes difficulties in manufacturing, and the finished product lacks flavor.

For better-grade crackers (standard two-inch-square size) the relative quantity of protein of 5.5% to 6.5% for the sponge and 8.5% to 9.5% for the total (sponge and dough) flours is recommended.

Soda ("free") causes off flavors if the pH is over 8.1 in the finished product.

Crackers made with a blend of spring and soft winter wheat flours, and experimentally made crackers, have the characteristics of hard-tack (crackers made from an unfermented dough).

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BLEACHING AGENTS VS. POTASSIUM BROMATE IN BAKING WITH DRY MILK SOLIDS [1]

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The use of bleaching agents is well established in the flour milling industry, as their correct use has been found to improve definitely the baking qualities of many wheat flours. The addition of potassium bromate, an oxidizing agent to most wheat flours, produces significant improvement in baking quality, it has been found. Many workers believe the effects of bleaching agents and of potassium bromate to be similar, although substantiating evidence for this belief has been lacking. One object of the present investigation has been to obtain information that would tend to prove or disprove this belief.

The baking industry during its rapid rise in the past half century has not overlooked the beneficial qualities of milk. As a result dry milk solids is now used extensively in bread-making formulas. Its use introduces problems to the commercial baker, many phases of which are

not completely understood. Ofelt and Larmour (1940) demonstrated that the use of dry milk solids tends to offset the harmful effect of adding excessive quantities of potassium bromate. In this investigation it was desired to study the effect of dry milk solids when used with flours given varied bleaching treatments or varied quantities of potassium bromate.

Review of Literature

It is not known when milk was first used as an ingredient in bread doughs but it is probable that housewives have included this ingredient in bread for many centuries. Milk in bread was studied from a nutritional viewpoint by Sherman *et al.* (1921), who found that it caused an improvement in growth and reproduction in rats when fed a simplified diet.

St. John and Bailey (1929a) demonstrated that the buffer action of dry milk solids was appreciable, as shown by the initial hydrogen-ion concentration of freshly mixed doughs and by the relative rates of change in the pH values of control and milk-containing doughs. These workers found that production of total carbon dioxide in yeast-leavened doughs was increased when dry milk solids was superimposed on the control formula. In a subsequent paper St. John and Bailey (1929b) observed that one weight unit of water was necessary with each added weight unit of milk to retain the same relative mobility of suspensions of flour, water, and dry milk solids. Skovholt and Bailey (1932) indicated that the inclusion of dry milk solids in dough increased the mixing time necessary to reach maximum plasticity, as shown by the farinograph.

The work of Amidon (1926) appears to be among the earliest dealing with the effects of dry milk solids on the baking quality of flours. This investigator found that dry milk solids produced an increase in loaf volume greater than the corresponding increase in dough weight. Crust color and character, break and shred, crumb color, flavor and taste, and texture were improved with increasing amounts of dry milk solids.

Skovholt and Bailey (1937) reported that dry milk solids had no effect on proteolytic activity. Diastatic activity was retarded by the introduction of dry milk solids, which reduced the hydrogen-ion concentration; gas production was accelerated; activity of the zymase complex was increased as a partial result of an effected reduction of hydrogen-ion concentration. Ofelt and Larmour (1940) observed that 6% of dry milk solids confers a high degree of tolerance to the action of bromate on flours, and gives optimum baking results.

Platey (1925) presented an excellent review of literature on the requirement uses of various commercial bleaching agents then in use.

Dedrick (1924) stated that too heavy a treatment of certain bleaching agents had been found to affect the strength and quality of the gluten. Overbleaching also tended to affect the color, imparting a yellowish or bluish gray tint to the flour as well as increasing the acidity of the flour. Agene improved the quality and gave greater loaf volume. Novadelox B bleached pure white, matured the gluten favorably, and did not over-bleach when excessive amounts were used. An increase in hydrogen-ion concentration of the flour was reported by Micros (1926), working with chlorine as the bleaching agent. Bleaching resulted in a shorter fermentation time, improved texture, and increased volume in the resultant loaf of bread.

According to Balls and Hale (1936) an optimum condition for proteolytic activity in the dough is absolutely necessary for best baking results. If, through overbleaching or overtreatment with chemical improvers, too many of the proteolytic enzymes have been destroyed, tough and unyielding doughs will be obtained, resulting in unsatisfactory bread. Smith (1937) reported that Novadelox had no discernible improving properties upon doughs, although baked loaves in many instances appeared to be improved in texture. Therefore, the question of overbleaching did not arise. Beta Chlora produced bulky and lively doughs, and loaves of better texture, color, and increased volume. With overtreatment by bleaching agents the doughs became short and lacked elasticity. Hanson (1932) found overbleaching with Beta Chlora detrimental to baking results.

Working (1928) explained the action of oxidizing agents on flour as a liberation of phosphatides which in turn reacted favorably upon the gluten. Geddes (1930) suggested that the benefits derived from the use of bromate may arise from the oxidation of certain germ components, presumably the phosphatides, believed to be detrimental. Attention was directed by Geddes and Larmour (1933) to a possible ionic effect by bromate on the gluten proteins whereby the gas-retaining capacities of the gluten were increased. In a series of papers, Jørgensen (1935a, 1935b, 1936) discredited the phosphatide and electrolyte theories and presented considerable data to support his contention that the benefits derived from bromate and other agents that behave in a like manner in bread doughs are the direct outgrowth of protease inhibition.

Methods and Materials

Ten-bushel lots of Tenmarq, Turkey, and Chiefkan wheats were selected on the basis of purity of variety, protein content, and test weight. The protein contents were approximately equal (Table I), thus making the samples desirable for a quality study from a varietal standpoint.

This flour protein range (12.2% to 12.6%) was desirable for the dry milk solids, bleaching, and potassium bromate investigations because it approximates the average range for many commercial flours from wheat grown in Kansas during 1939.

TABLE I

ANALYTICAL DATA FOR TEN-BUSHEL LOTS OF WHEAT AND
FLOURS MILLED THEREFROM

Variety	Test weight of wheat	Moisture		Protein[1]		Ash[1]		Baking absorption[1]
		Wheat	Flour	Wheat	Flour	Wheat	Flour	
	lbs.	%	%	%	%	%	%	%
Tenmarq	59.2	10.4	12.7	13.6	12.2	1.234	0.399	61.6
Turkey	56.7	10.5	13.3	13.6	12.5	1.748	.441	60.8
Chiefkan	58.9	11.9	13.3	13.8	12.6	1.852	.412	62.7

[1] Moisture basis 15%.

The wheat samples were milled on the Kansas State College commercial mill. After milling the unbleached flours were placed immediately in a cold room held at approximately 41°F. Special apparatus was constructed to bleach small samples of flour with gas.

Agene, Beta Chlora, and Novadelox were used as bleaching agents in amounts of ½, 1 (full), 1½, 2, 3, and 4 times the bleach used by many commercial mills as normal for straight-grade flour. The amount of bleach added to each sample was based upon the following schedule as the normal of full bleach: Agene, 3.0 g. per barrel of flour (196 pounds); Beta Chlora, 0.75 oz. per barrel of flour; Novadelox, 0.40 oz. per barrel of flour. One-pound samples were bleached and stored in tightly covered cans at room temperature (70°F.) for 10 days. In order to reduce possibilities of error in bleaching, carotene ($C_{40}H_{56}$) determinations were made on the flours by the method of Binnington and Geddes (1939) and photometric apparatus described by Shrewsbury, Kraybill, and Withrow (1938).

The baking formula used was as follows:

Flour	200 g.
Water (distilled)	As required
Yeast	4 g.
Sugar	12 g.
Salt	3 g.
Shortening (hydrogenated vegetable)	6 g.
Malt syrup (120°L.)	0.5 g.
Dry milk solids	As indicated
Potassium bromate	As indicated

When used, dry milk solids was added at the 6% level in accordance with the findings of Ofelt and Larmour (1940). Absorption values for milk-

containing doughs were increased over milk-free doughs 1% for each 1% of dry milk solids used.

Doughs were mixed at 66 rpm. to optimum consistency determined by observation in a Swanson-Working mixer, using a bowl containing four pins. Mixing time and absorption were held constant for each variety. Doughs were divided into two equal portions after mixing, fermented and proofed at 86°F. and 90% relative humidity.

Fermentation was as follows:

First punch after	105 min.
Second punch after	50 min.
Mold after additional	25 min.
Total	180 min.

A National " pup " sheeting roll was used for punching, while molding was done on a Thompson Model A laboratory molder. The loaves were baked at 430°F. for 24 minutes in a specially designed Despatch oven (Finney and Barmore, 1939). Loaf-volume figures are averages of at least four loaves from two bakes on different days. Some mixes were triplicated when necessary. To be considered satisfactory the two loaves obtained from one mix did not differ more than 25 cc. in volume. When the average of loaves from different mixes agreed within 20 cc. it was considered a satisfactorily checked result. Loaves were cut the following morning to grade internal characteristics and to obtain photographs for a permanent record. In calculating the total baking scores the loaf characteristics were weighted after the method of Barmore, Finney, and McCluggage (1940) in the following manner:

$$\text{Loaf volume score} = (\text{actual loaf volume} - 300 \text{ cc.}) \times 0.1$$
$$\text{Grain score} = (\text{score on basis of } 100) \times 0.3$$
$$\text{Texture score} = \text{score made on basis of } 22 \text{ points}$$
$$\text{Color score} = (\text{score on basis of } 100) \times 0.1$$

Experimental Data

The optimum bromate requirements for the three flours are given in Table II. The baking results and carotene determinations are shown in Tables III to V and photographs of representative loaves are shown in Figures 1 and 2. Figures 3, 4, and 5 show loaf volumes and carotene values graphically.

TABLE II

OPTIMUM $KBrO_3$ REQUIREMENTS OF THREE FLOURS WHEN BAKED WITH AND WITHOUT 6% DRY MILK SOLIDS (DMS)

	Tenmarq	Turkey	Chiefkan
	mg.	*mg.*	*mg.*
No milk	2	2	2
With DMS	4	6	4

TABLE III

DATA ON TENMARQ FLOURS GIVEN VARIOUS TREATMENTS AND BAKED WITH OR
WITHOUT 6% DRY MILK SOLIDS (DMS)

Treatment	Carotene content	No milk		6% DMS	
		Loaf volume	Total baking score	Loaf volume	Total baking score
	ppm.	*cc.*		*cc.*	
NO TREATMENT					
None	1.64	620	80.1	736	92.7
AGENE (*g. per bbl.*)					
1.5	0.64	660	87.1	770	98.2
3.0	0.40	663	86.7	778	101.2
4.5	0.24	666	88.0	792	102.4
6.0	0.20	670	88.9	796	103.2
9.0	0.14	682	89.3	790	105.3
12.0	0.06	690	90.4	802	105.1
BETA CHLORA (*oz. per bbl.*)					
0.38	0.92	633	80.6	750	95.4
0.75	0.68	620	79.8	724	93.2
1.13	0.60	621	81.5	735	94.7
1.50	0.52	634	85.1	735	96.0
2.25	0.51	668	87.8	745	98.5
3.00	0.50	665	86.3	756	100.6
NOVADELOX (*oz. per bbl.*)					
0.20	1.09	618	79.3	738	95.0
0.40	0.75	633	81.9	747	97.5
0.60	0.49	648	83.6	749	98.6
0.80	0.34	655	82.8	757	98.8
1.20	0.20	656	85.2	771	101.7
1.60	0.16	668	89.5	791	104.3
KBrO$_3$ (*mg. per 100 g. flour*)					
1	—	729	96.6	852	106.8
2	—	745	95.9	864	113.2
3	—	723	92.0	870	114.5
4	—	698	90.1	912	119.9
5	—	667	88.3	899	116.2
6	—	650	84.9	858	112.2
7	—	—	—	852	111.8

TABLE IV

DATA ON TURKEY FLOURS GIVEN VARIOUS TREATMENTS AND BAKED WITH OR WITHOUT 6% DRY MILK SOLIDS (DMS)

Treatment	Carotene content	No milk		6% DMS	
		Loaf volume	Total baking score	Loaf volume	Total baking score
	ppm.	*cc.*		*cc.*	
NO TREATMENT					
None	2.55	607	72.8	638	68.9
AGENE (*g. per bbl.*)					
1.5	1.68	607	73.4	650	74.0
3.0	0.97	616	73.7	652	76.5
4.5	0.76	614	74.7	654	78.5
6.0	0.70	620	79.9	665	81.0
9.0	0.54	630	81.9	664	81.8
12.0	0.42	628	82.4	678	86.0
BETA CHLORA (*oz. per bbl.*)					
0.38	1.81	608	72.4	632	71.7
0.75	1.40	618	76.4	622	73.1
1.13	1.24	628	76.4	627	73.5
1.50	1.15	626	76.3	627	76.0
2.25	1.11	630	80.4	626	74.0
3.00	1.07	646	81.9	638	76.0
NOVADELOX (*oz. per bbl.*)					
0.20	1.78	617	74.8	648	72.6
0.40	1.20	616	74.0	655	77.8
0.60	0.81	628	78.9	649	77.2
0.80	0.71	625	77.9	663	80.6
1.20	0.34	635	81.0	657	82.1
1.60	0.23	651	83.9	673	85.1
KBrO₃ (*mg. per 100 g. flour*)					
1	—	751	97.6	739	96.8
2	—	840	107.0	788	101.9
3	—	818	104.4	838	109.2
4	—	816	102.3	879	114.3
5	—	762	97.0	915	117.3
6	—	760	95.4	932	119.0
7	—	—	—	884	115.3

TABLE V

DATA ON CHIEFKAN FLOURS GIVEN VARIOUS TREATMENTS AND BAKED WITH OR
WITHOUT 6% DRY MILK SOLIDS (DMS)

Treatment	Carotene content	No milk		6% DMS	
		Loaf volume	Total baking score	Loaf volume	Total baking score
	ppm.	*cc.*		*cc.*	

NO TREATMENT

None	2.07	557	62.1	606	66.4

AGENE (*g. per bbl.*)

1.5	1.29	580	66.2	635	72.9
3.0	0.79	580	68.6	637	75.5
4.5	0.67	581	70.0	638	76.0
6.0	0.65	591	72.6	639	79.7
9.0	0.61	590	75.9	642	79.7
12.0	0.53	605	79.2	642	80.5

BETA CHLORA (*oz. per bbl.*)

0.38	1.38	577	66.6	617	69.5
0.75	1.17	587	69.3	624	72.9
1.13	1.15	587	70.7	616	72.1
1.50	1.13	585	72.0	614	74.2
2.25	1.01	595	76.2	615	74.0
3.00	0.96	605	78.2	620	76.2

NOVADELOX (*oz. per bbl.*)

0.20	1.18	570	66.6	625	73.5
0.40	0.80	575	67.5	624	75.7
0.60	0.51	584	70.2	627	76.1
0.80	0.39	592	71.5	639	78.3
1.20	0.31	608	73.0	640	78.2
1.60	0.30	608	72.2	645	78.7

KBrO$_3$ (*mg. per 100 g. flour*)

1	—	613	78.3	639	79.8
2	—	652	86.9	685	86.7
3	—	634	86.3	702	94.1
4	—	628	84.6	712	95.7
5	—	618	82.6	712	94.2
6	—	573	79.1	712	95.0
7	—	—	—	712	95.0

Discussion

The data show that the presence of 6% dry milk solids tends to increase the loaf volume. Tolerance to potassium bromate was obtained through the use of dry milk solids. This suggests that dry milk solids in baking act as a buffering agent. Table V and Figures 2 and 5 show that this buffering effect was most noticeable in Chiefkan. Table II indicates that the use of dry milk solids increased the bromate needed for optimum loaf volume and characteristics in all three varieties. The crust color, break and shred, general appearance of the loaf, and grain and texture were improved by the addition of dry milk solids. It was found easier to check duplicate bakes when the formula included dry milk solids, thus giving evidence that more uniform loaves could be obtained through the use of this milk product.

Four times the average bleach was employed experimentally to determine whether such treatment would cause the flour to break down as it does when an excessive amount of potassium bromate is added. A gradual increase in loaf volume and total baking score indicated that the bread was improved by additions of Agene and Novadelox. Beta Chlora gave the only evidence of overbleaching, which occurred with the intermediate treatments. Without dry milk solids this overbleaching was more pronounced. There was evidently more buffering effect from dry milk solids when Beta Chlora was used than with potassium bromate. The fact that more chlorine, which has definite acidic properties, was used in making a normal treatment of Beta Chlora than a four-normal treatment of Agene may account for the difference in the behavior of the two bleaches. Novadelox, not generally considered as a flour improver, was on a par with Agene in aging effect upon the flour. There is no evidence here as to whether this improvement is a direct result of gluten development by Novadelox, or a result of the filler used in Novadelox acting as yeast food, or a combination of both.

The doughs became tighter as the amount of potassium bromate or bleaching agent added was increased, giving evidence that these treatments increased the water absorption. The use of optimum amounts of potassium bromate produced larger loaves and better bread than any bleaching treatment. It appears that " oxidation " by bleaching and " oxidation " by potassium bromate may be of a different type and the terms should not be used synonymously.

The series of flours used in this study provided an excellent basis for variety comparisons. It can be readily seen that bleached Tenmarq without dry milk solids was superior to Chiefkan with or without dry milk solids. This was also true when the optimum amount of potassium bromate was used. The superior gluten quality of Tenmarq as
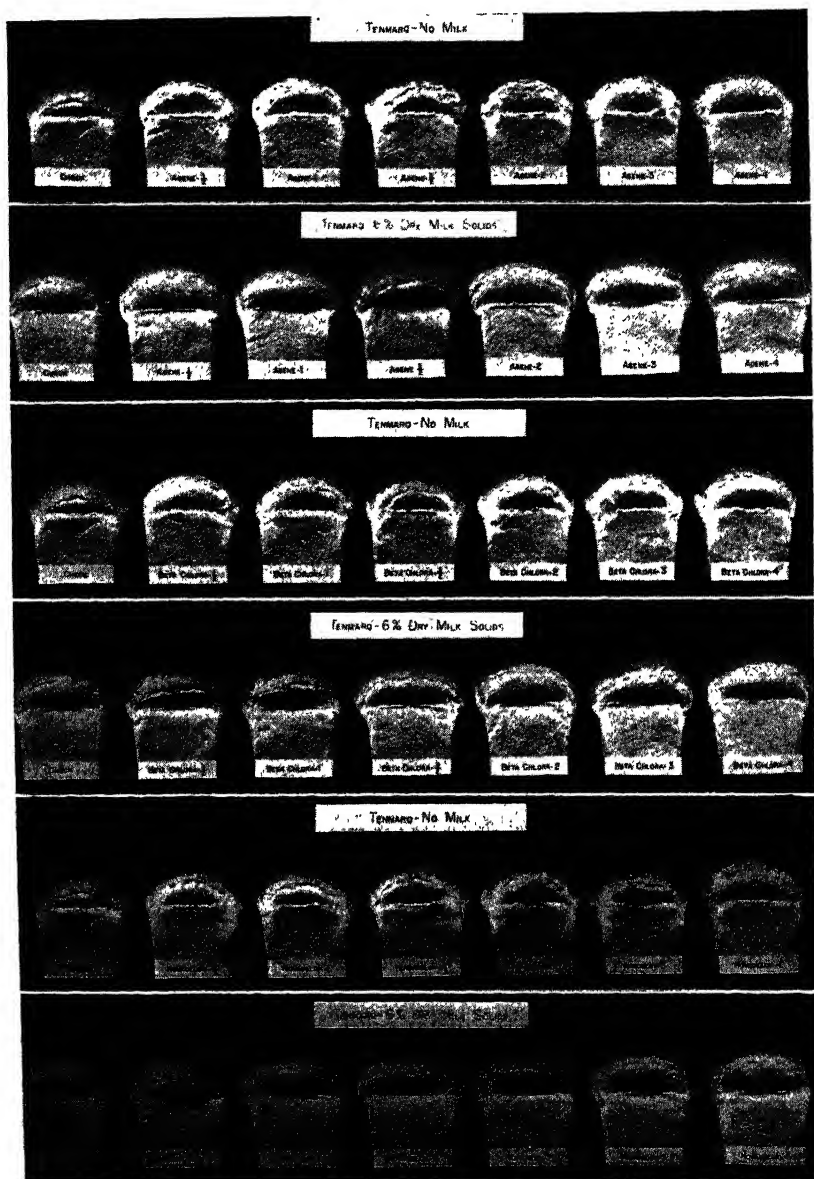
Fig. 1. Bleached Tenmarq flours baked with and without dry milk solids.
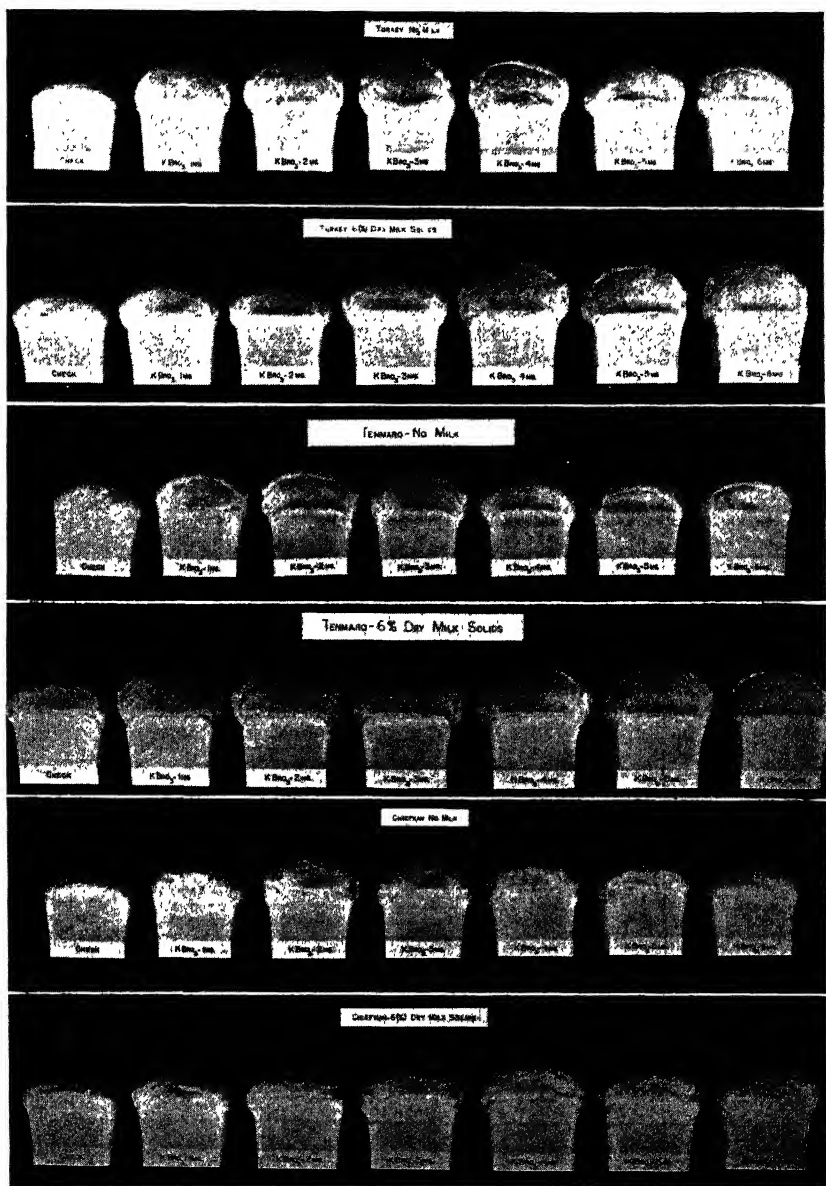
Fig. 2. Loaves baked with various amounts of potassium bromate.
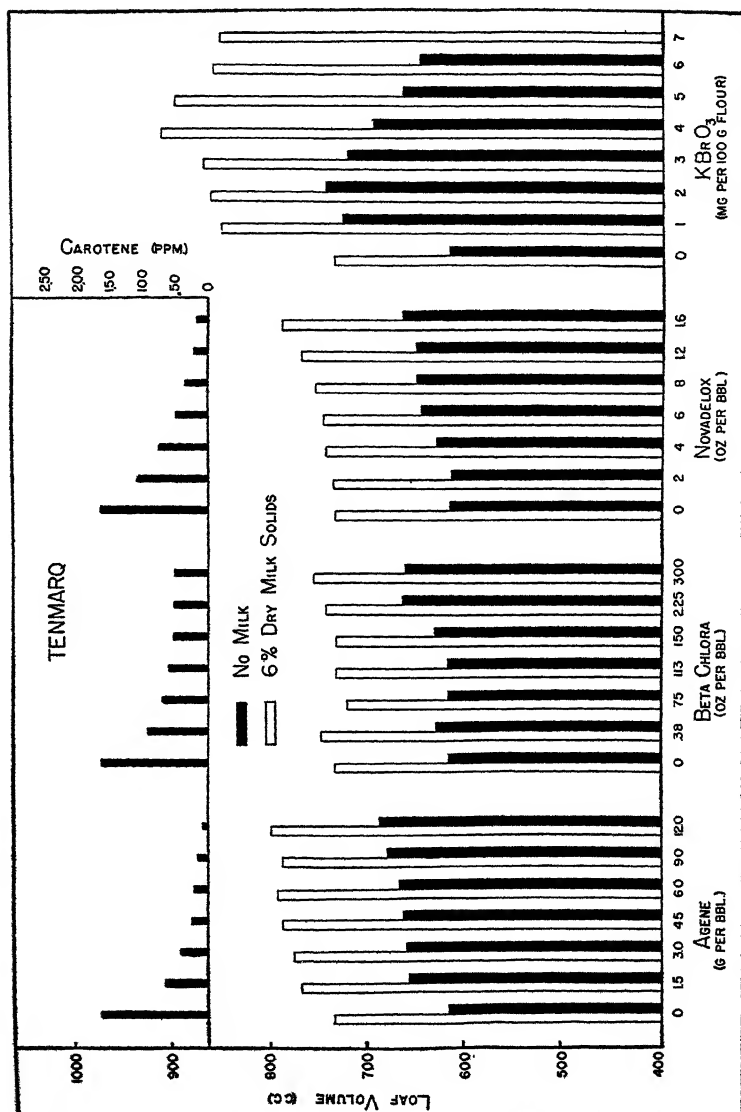
Fig. 3. Variations in loaf volume as influenced by bleaching agents, potassium bromate, and dry milk solids. The effect of various kinds and rates of bleach upon carotene content is also shown. Variety of wheat: Tennarq.
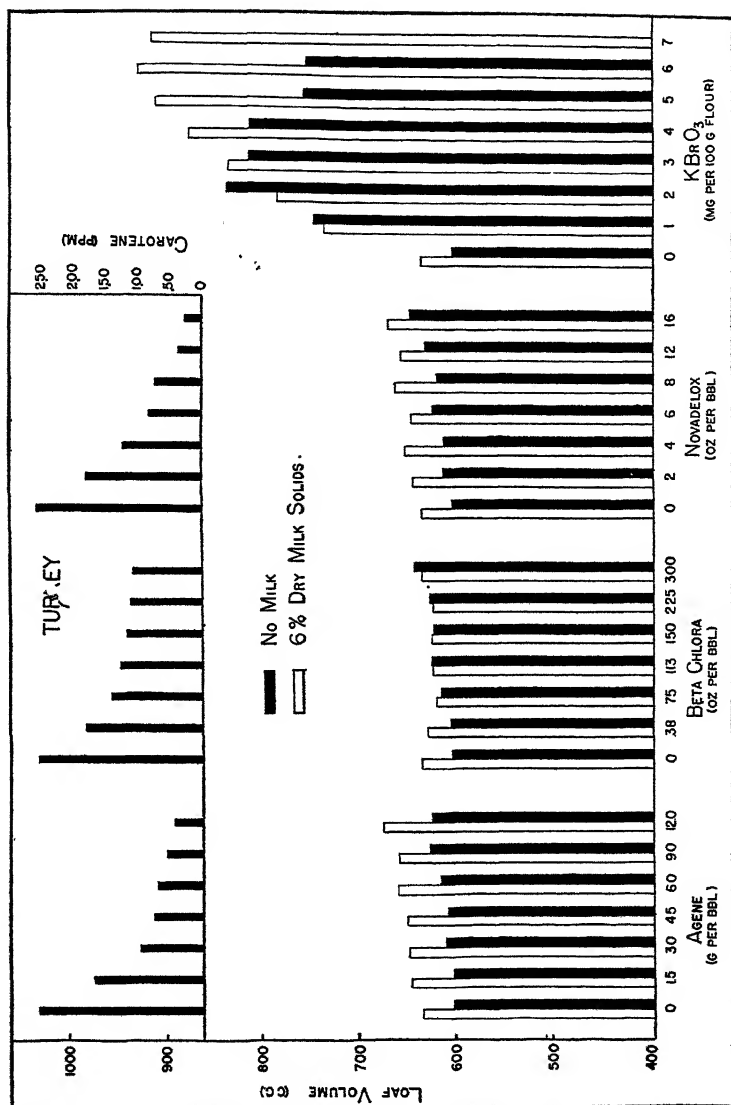
Fig. 4. Variations in loaf volume as influenced by bleaching agents, potassium bromate, and dry milk solids. The effect of various kinds and rates of bleach upon carotene content is also shown. Variety: Turkey.
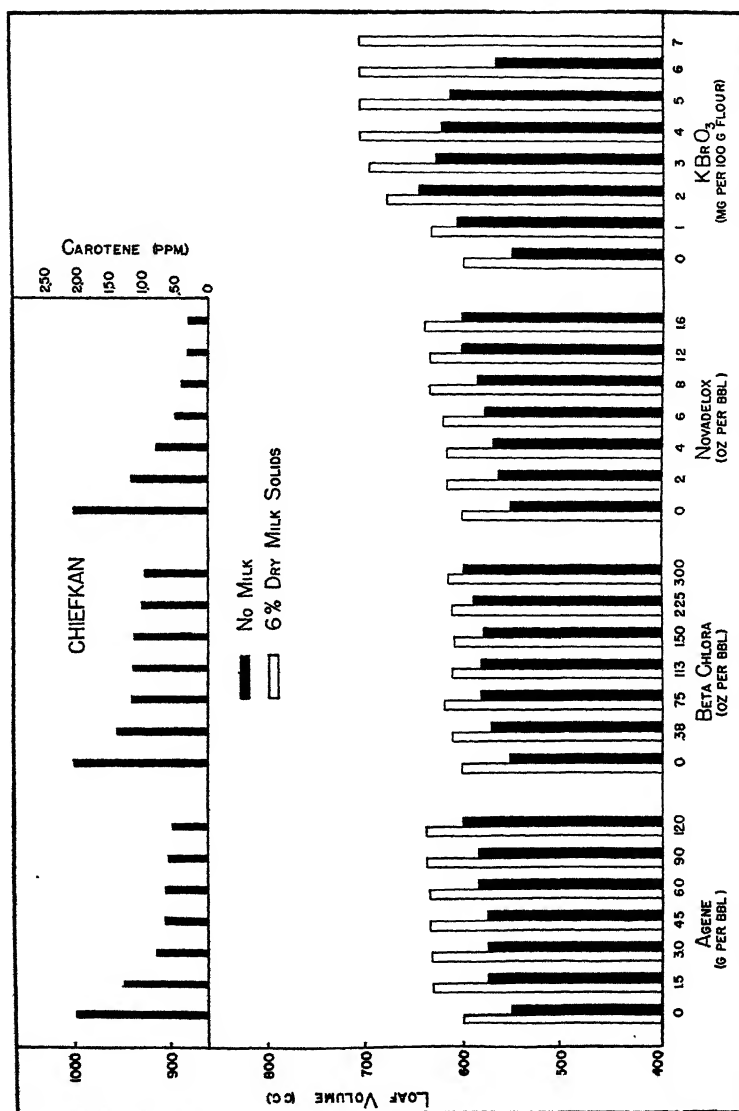
Fig. 5. Variations in loaf volume as influenced by bleaching agents, potassium bromate, and dry milk solids. The effect of various kinds and rates of bleach upon carotene content is also shown. Variety: Chiefkan.

compared to Chiefkan apparently enables it to respond more as a result of adding bromate or dry milk solids in spite of the fact that the Chiefkan sample was higher in protein than the Tenmarq. Turkey definitely had a high requirement for potassium bromate with milk, while Tenmarq did not (Table II). Turkey did not respond relatively as much to bleaching as did Tenmarq. Turkey responded more than Tenmarq to potassium bromate. The latter variety gave better dough development with potassium bromate than with bleaching. Tenmarq definitely displayed the greatest improvement upon the addition of dry milk solids. Chiefkan with dry milk solids possessed a greater tolerance to bromate than any of the varieties investigated. The Tenmarq and Turkey total bread scores at optimum bromate levels with dry milk solids differed only by two points.

Carotene determinations showed that carotene content varied inversely as the amount of bleach applied. These determinations were made to insure the accuracy of results with the bleaching apparatus. Upon this basis the apparatus indicated that it was capable of accurately bleaching one-pound samples of flour with gaseous bleaches.

There was variation in the carotene content of varieties. Unbleached Tenmarq had the least carotene, followed by Chiefkan, while the greatest amount was found in Turkey. However, carotene content decreased relatively the same for all varieties as the amount of bleach increased. As the higher treatments were reached there was a tendency for the carotene content to decrease less rapidly with increasing quantities of bleach. With normal treatments flour and crumb colors were of such a nature that the effect of higher treatments was not easily noticeable.

Beta Chlora was less effective in bleaching the carotene in all flours than was Agene or Novadelox, the latter two being quite comparable in their results.

That the effect of bleaching agents was quite different from that of potassium bromate was evidenced by the increased whiteness in crumb color of bread from bleached flours. Bread made with unbleached flour but with added potassium bromate showed a more yellow color in the crumb than did the bread from bleached flours. This observation further differentiates between bleaching and bromate action and further indicates that the terms should not be used synonymously.

It was evident that flour doughs reacted differently to treatment with bleaching agents and treatment with potassium bromate. Bleaching agents had a notable effect upon the coloring matter of flour, principally carotene, making it colorless. Some effect upon the gluten and baking properties was also evident. The crumb color was whiter, and the volume, grain, and texture were noticeably improved. Potassium bro-

mate, on the other hand, had no appreciable effect upon the coloring matter. It did, however, increase the loaf volume and improve the grain and texture to a much greater extent than did bleaching agents. It is believed from this that potassium bromate acted mainly upon the gluten proteins. This, however, does not eliminate the possibility of its action upon the phosphatides (Working, 1928) nor its inhibitive action upon the proteases (Jørgensen, 1936).

Summary and Conclusions

A study was made of the effects of commercial bleaching agents and of potassium bromate upon the color and baking characteristics of flours from Tenmarq, Turkey, and Chiefkan wheats. In addition, the effect of dry milk solids was studied after the flours were given varied bleaching treatments as well as varied quantities of potassium bromate.

Dry milk solids, when included in the baking formula, was used at the 6% level. The flours were baked with amounts of potassium bromate ranging from 0 to 7 mg. per 100 g. of flour. Agene, Beta Chlora, and Novadelox were the commercial bleaches used in amounts ranging from zero to four times the normal treatment.

The results obtained clearly indicated that the presence of 6% dry milk solids conferred a certain degree of tolerance toward potassium bromate, increased the bromate requirement, increased the loaf volume, and improved the grain, texture, and crust color.

Six percent of dry milk solids increased the loaf volume and improved the grain and texture, crust color, and break and shred of loaves baked from bleached flours.

The bread was gradually improved by the addition of increasing amounts of Agene and Novadelox. Beta Chlora at intermediate treatments gave smaller loaf volumes, with a trend toward increased loaf volumes at heavier treatments.

The carotene content of the flours varied inversely with the amount of bleaching agent used.

The effect of bleaching agents and the oxidizing effect of potassium bromate should not be considered the same, because in no case was the loaf volume or total bread score from bleached flours comparable to that obtained with potassium bromate at the optimum level. Bleaching whitened the crumb color; potassium bromate did not.

Tenmarq and Turkey proved to be superior to Chiefkan in baking quality. Turkey responded the most to potassium bromate. Tenmarq had the least carotene content of the three varieties and responded the most to bleaching treatment as well as to added dry milk solids.

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FACTORS AFFECTING THE SOLUBILITY OF CORN PROTEINS [1]

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As part of a study of corn genetics, it is desirable to analyze for the constituent proteins of the corn kernel. According to Showalter and Carr (1922), Spitzer, Carr, and Epple (1919), and Zeleny (1935), the ratio of the salt, alcohol, and alkali-soluble proteins present in corn varies with the type or strain of corn and the age of the kernel. From the results of a number of analyses it became evident that large variations in the apparent percentages of the different fractions were caused by relatively slight alterations in the procedures used for the extractions. The purpose of this manuscript is to report some of the factors affecting the peptization of corn proteins during such a fractionation.

Since Osborne's early work on the extraction of proteins, evidence has accumulated which shows that the factors determining protein solubility are very diverse and complex. Approaches from the theoretical side, such as those made by Cohn (1938), indicate that the solubility of amino acids (and proteins) in water is influenced by the presence of other amino acids and by salts, with the latter possessing both solvent and salting-out properties, depending upon the nature and the concentration of the salt used.

The extensive investigations of the wheat proteins by Sinclair and Gortner (1933), Blish and Sandstedt (1925), and McCalla and Rose (1935) suggest that the separation of the wheat proteins into fractions soluble in salt, alcohol, and alkali solutions by the Osborne method is largely superficial. Thus most or all of the wheat protein may be peptized by salts such as potassium iodide and sodium salicylate; and, conversely, when the protein is peptized in alcoholic alkali it cannot be neutralization be separated into fractions corresponding to those obtained by direct extraction. Lyotropic studies by Staker and Gortner (1931),

The product swelled slightly in water, but appeared to be rather completely retrograded.

Nothing could be centrifuged at 2400 rpm. in 30 minutes from the beta-amylose solutions within the intervals given, although they did become very slightly opalescent almost as soon as they were neutralized. After the first 24 hours a small but definite amount of material began to flock out of the beta-amylose solutions, more noticeable in the one at pH 3.8. However, even up to 48 hours the quantity was too small to isolate and weigh.

In the section that follows additional proof will be offered that gamma-amylose is not formed by the association of the common amylose units of starch, nor by the obscure mechanism of carbohydrate dehydration, nor by any other process that is in accord with theories commonly used to explain the phenomenon of retrogradation.

Gamma-Amylose as a Product of Synthesis by Diastase

In this section we will deal with the viewpoints of those chemists who, while not necessarily subscribing to theory No. 2, are nevertheless skeptical of separation procedures involving enzymolysis, on the ground that the end products may be the results of synthesis during the course of

TABLE III

DECREASE IN WEIGHT OF INSOLUBLES FROM CORN STARCH AFTER 40 HOURS'
DIGESTION WITH BARLEY DIASTASE

Additional hours of conversion	Digestion continued with:	
	Malt diastase	Barley diastase
	g.	g.
0	0.1349	0.1349
68	0.0302	0.0352
120	0.0120	0.0170

the reaction. Synthesis, for example, probably occurs in the extended acid hydrolysis of starch. After sufficient dextrose builds up in solution it is polymerized into various sugars whose configurations may not have existed originally in the starch. The processes are explainable on a physical-chemical basis of equilibria. It was to anticipate this argument that after an initial enzymolysis in our procedures, the maltose, dextrins, and other soluble products of reaction were washed out and our product converted a second time with an excess of barley diastase.

In preparation No. 7, the enzyme treatment was extended after purification of the product by adding fresh enzyme two additional times. In each case there was a gradual decrease in the amount of insoluble gamma-amylose resulting, indicating that the product is slowly hydro-

lyzed and not synthesized by barley diastase. A parallel experiment indicates moreover that the product is digested at a slightly faster rate by an equivalent amount of malt diastase.

The experiment shows, incidentally, that our method is unsuitable for a quantitative estimation of gamma-amylose, since a small part of the product must be solubilized during the course of preparation by barley diastase, especially when alkali-gelatinized starch is used in which the gamma-amylose is evenly dispersed and rendered more accessible to the enzyme.

Returning to the question of synthesis, it is conceivable that a synthesis may result that is not dependent on a reversible equilibrium. For example, the synthesis of the Schardinger dextrins is not a reversible equilibrium inasmuch as we have observed that once these dextrins are formed and isolated, a fresh supply of the amylase from *B. macerans* added to this product does not result in further change. But it is noteworthy that, given the basic products to work with, *B. macerans* is capable of producing the dextrins, whether the source be potato, tapioca, or corn starch.

Thus it seemed advisable to test the synthesis theory for gamma-amylose by allowing barley diastase to work on both tapioca and potato starch. Using our procedures (omitting dioxane extraction of the starch), a yield of only 0.52% was obtained from tapioca, and a yield so small for potato starch, 0.094%, that some doubt exists as to whether gamma-amylose is actually a constituent of potato starch or, rather, an impurity resulting from incomplete removal of some material during milling (Ling, 1928). If " starch molecules " are more or less the same, then it has been shown beyond doubt that gamma-amylose cannot be a product of synthesis. Otherwise, under strictly comparable conditions, substantially the same yields of gamma-amylose would have been obtained from tapioca and potato starch that were obtained from corn starch.

The above experiment also indicates that our product does not result from association of beta-amylose units into an insoluble form, nor is it the end product of any other mechanism used to explain retrogradation. In fact it is apparent from the nature of our procedure that beta-amylose could not be expected to have retrograded during the operations used. We purposely alkali-gelatinized the starch to put into solution any naturally occurring retrograded amylose; we used higher temperatures for conversion (55°C.) than were used by Ling or recommended by Tychowski for barley diastase conversion; and we neutralized our alkali gelatinization only to pH 6.2, thereby providing conditions that are very nearly ideal for keeping starch pastes over long periods of time with a negligible formation of reversion products. This was strikingly con-

firmed in preparing gamma-amylose from tapioca starch. As long as the conditions of our procedures were followed, only very small quantities of insolubles resulted, and these proved to be gamma-amylose. However, in subsequent tests wherein we attempted to shed more light on the meager yield from tapioca starch, we made one conversion at 20°C. The solution, very nearly clear at the start, clouded up considerably in 24 hours, and after several days' standing surprisingly large quantities of insolubles could be separated. This mass, however, proved to be a product of reversion, quite similar to retrograded amylose.

However, some investigators have noted the extent and speed with which corn-starch pastes cloud up on cooling, throwing out apparently more insoluble material than corresponding pastes from potato or tapioca starch, and from the assumption that the above is a retrogradation effect, they may be inclined to propose that the corn-starch molecule, or amylose, is more predisposed to retrogradation than are tapioca or potato amyloses. They should first explain what causes the corn-starch molecule to be more predisposed to retrogradation, if indeed all starch molecules are fundamentally the same. As against the foregoing assumption it is noteworthy that with 5%–10% pastes made by alkali-gelatinizing dioxane-extracted corn, tapioca, and potato starch, the relative cloudiness which immediately results from acidification to pH 5.0 stands in qualitative relationship, at least, to the relative yields of gamma-amylose obtained from these pastes 24 hours later. The fact that this precipitation occurs immediately and abundantly with corn starch argues rather that the factor responsible for cloudiness and precipitation is gamma-amylose, not retrograded beta-amylose. In conclusion, the supposition above given neglects the possibility that in corn starch there exists a component, soluble with difficulty in water at room temperature but whose solubility is materially increased at higher temperatures.

That the phenomenon discussed is not retrogradation is further confirmed by the extreme ease with which these alkali-gelatinized corn-starch pastes are converted by diastase, which we will demonstrate in the second paper of this series, and wherein we differentiate gamma-amylose from amylo-pectin. Thus it is concluded that gamma-amylose is neither a product of reversion nor the product of any other secondary reaction occurring in the presence of barley diastase.

Quantitative Determination of Gamma-Amylose in Corn Starch

Having reported on such properties of gamma-amylose as its solubility and susceptibility to enzyme attack, it is in order to discuss an attempt to determine quantitatively the gamma-amylose content of corn starch.

Fifteen grams of dioxane-extracted corn starch was treated in a 250-cc. centrifuge bottle by a method similar to that used in isolation procedures, except that the enzyme-to-starch ratio was increased 25%. The bottle was observed during enzymolysis for the purpose of noting the first point at which the insolubles could be quantitatively centrifuged out at 2500 rpm. in 20 minutes. This occurred after about 5 hours' conversion time at 50° to 55°C. The insolubles were thoroughly washed in the centrifuge until chloride-free, without transfer from the original bottle. After the third wash water it was necessary to add ethyl alcohol to the water to facilitate the complete centrifuging of the product. After washing, it was quantitatively transferred to an evaporating dish and the dry solids were determined. The centrifugate from the conversion was allowed to stand 24 hours at 5°C. Nothing further was precipitated.

TABLE IV

YIELD OF GAMMA-AMYLOSE FROM CORN STARCH AT VARIOUS
CONVERSION TIMES

Conversion time, barley diastase at 50°–55°C.	Yield (dry basis)
hrs.	%
0	(Estimated 9.15)
5	8.09
8	7.55
16	6.12
24	4.97

Similar conversions were made, extending the conversion time to 8, 16, and 24 hours respectively. The percentage of gamma-amylose obtained at these various times of conversion are given in Table IV.

To estimate the amount of gamma-amylose present in corn starch originally, we plotted the logarithms of the yields obtained against conversion times and extrapolated to zero time (Figure 1). The original content by this method was determined as 9.15%. We are inclined to believe therefore that approximately 10% of the corn-starch granule consists of gamma-amylose.

Gamma-Amylose as a Higher Polymer of Amylose

It is the purpose in this section to consider the possibility that there may be modifications of soluble amylose other than those considered, as for example higher orders of polymerization which would account for the greater resistance and lesser solubility of the component separated by us as gamma-amylose. On the basis of this theory, gamma-amylose should be among the highest. Accordingly, the acetyl derivative of the latter was prepared and studied (see " Experimental " section).
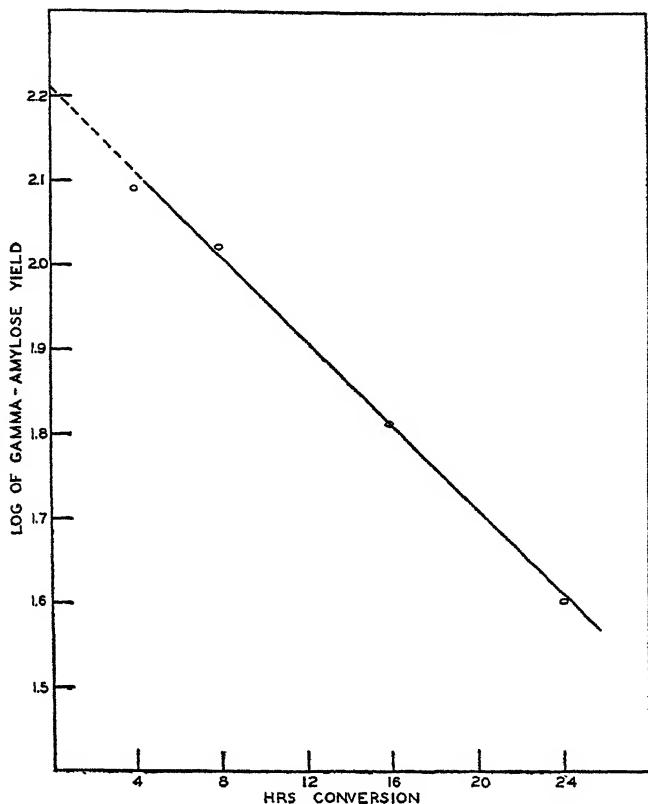
Figure 1.

It may be recalled that the weight of evidence now leads to the conclusion that starch products may be acetylated and deacetylated, under certain conditions, with no change in the degree of polymerization, although Richardson, Higgenbotham, and Farrow (1935) several years ago indicated that this point was controversial. Pringsheim and Persh (1922) and also Pringsheim and Dernikos (1922) pointed out that the Schardinger dextrins from starch could be acetylated in pyridine with acetic anhydride with no change in molecular size, as evidenced by molecular-weight determinations by crysoscopic methods. More recently Staudinger (1937) confirmed the fact that there was no change in the degree of polymerization of various starches during acetylation in pyridine and deacetylation, using osmotic pressure and specific rotation in formamide as a basis of judgment. Finally Higgenbotham and Richardson (1938) adopted the view that the starch molecules remained intact

during acetylation in pyridine since upon deacetylation the product showed the same copper reduction value as the original product before acetylation.

Accordingly, gamma-amylose was acetylated by the acetic anhydride-pyridine method using procedures substantially like those used by Cantor (1934) for acetylation of carbohydrate mixtures. His procedures were selected for the reason that they were shown to involve a minimum of degradation and of rearrangement of the component saccharides (see " Experimental " section).

Acetyl groups, determined by Freudenberg's transacetylation method, were found to be 20.3%, indicating a monacetate. However, the unreliability of this method for the acetates of higher carbohydrates is sug-

TABLE V

OPTICAL ACTIVITY OF GAMMA-AMYLOSE 6-A

Product	Solvent	$(\alpha)_D^{25}$
Potato starch, purified	0.05N NaOH	154.0
Gamma-amylose	0.05N NaOH	135.0
Gamma-amylose	24 hrs. in 0.05N NaOH	135.0
Gamma-amylose	0.10N NaOH	134.8
Gamma-amylose	Triton B	135.7
Gamma-amylose	Heated to dissolve in formamid	133.0
Gamma-amylose	Treated as in deacetylation, 0.1N NaOH	131.6
Acetyl gamma-amylose	Deacetylated 0.1N NaOH	132.5
Maltose (Pfanstiehl)	0.05N NaOH	115.0

gested by contrasting this figure with the value obtained by the use of the method of Murray, Staud, and Gray (1931), whereby acetyl groups found were 44.1% and 44.5%, against a theoretical for a triacetate of 44.8%.[2]

A molecular weight of 4,890 ± 390 was found by depression of the freezing point of camphor, which for a triacetate indicates a chain length of 17 ± 2 dextrose units. This result does not substantiate the supposition that gamma-amylose is composed of polymers of a higher order than the commonly occurring amylose units in starch, especially when considered in the light of the estimates of chain length for starch (given in recent years) ranging from 24 to 30 dextrose units (as found by Haworth and Hirst, 1933) up to about 1,000 units (as found by Richardson, Higginbotham, and Farrow, 1936). Indeed, from these modern estimates of the average chain length of starch, the gamma-amylose fraction should be the shortest constituent and could not, therefore, be a polymer of the more frequently occurring beta-amylose.

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[2] This analysis was performed by Dr. T. J. Schoch, who has pointed out the unreliability of the Freudenberg method.

Gamma-amylose was deacetylated by prolonged stirring in dilute alkali and the optical rotation determined on the solution at 25°C. For comparison the optical activity of gamma-amylose, 6–A, treated in identical fashion with alkali, is also given in Table V, as well as other pertinent specific rotations.

Several points can be made from the above data.

First, the observation that acetylation procedures such as those used by us involve no perceptible degradation of the carbohydrate can be confirmed. There is, however, a noticeable degradation of the gamma-amylose in prolonged contact with alkali as high as or higher than $0.5N$ even at room temperature, and indeed a measurable one at lower alkalinities if heat is applied (as with formamid). Probably the lower rotation value obtained for gamma-amylose after deacetylation of the acetate is the result of the action of the alkali used in deacetylation.

Secondly, our above-given observation that gamma-amylose is not of greater molecular magnitude than the other more commonly occurring amyloses is confirmed by the fact that the specific rotation of the former is midway between that for maltose and potato starch, the latter starch containing no gamma-amylose.

Third, there is evidence to the effect that our product is uniform. If it is, however, a mixture or series of dextrose polymers (such as a homologous series) rather than one individual molecule, then the variation in molecular size between the highest and lowest members is apparently small.

Gamma-Amylose as a Radical on the Corn Starch Molecule

Those who subscribe to the last of the four theories mentioned early in this paper would doubtless explain the difference between corn starch and potato starch by supposing that gamma-amylose exists in the former (but not in the latter) as a complex radical that is hydrolyzed off by the diastase. But how can they explain the mechanism of this diastatic splitting, since obviously the radical must be attached to one of the ends of the main chain, or to the free end of one of the side chains, or at some intermediate point? The diastase must work gradually up to this point of attachment, rather than to start its hydrolysis by a haphazard attack on some glucosidic chain linkage, which would in all probability form dextrose and maltose, mole for mole. Furthermore, if configurations such as those proposed by Staudinger are to be taken seriously, then rather appreciable amounts of disaccharides other than maltose should also be produced even with beta-amylase. Thus the modern conception is that only the maltose configuration (or some labile configuration that

immediately arranges to maltose after conversion [3]) fits the barley dias-
tases and, in proceeding from a free end, conversion proceeds until a
configuration is encountered which is not that of maltose. Hence a
branch in the chain or some other anomaly would present a barrier to
further enzymolysis as has been suggested by Myrbäck (1939).

If the gamma-amylose radical is attached to the number one carbon
of the potential aldose group of the main chain, then for the diastase to
reach its point of attachment the chain must be perfectly straight (con-
sist of only maltose groups), and maltose should be the only other prod-
uct of hydrolysis. The supposition is not granted since the corollary is
not in accord with the facts.

If the radical is attached to any other terminal, then the enzyme must
start hydrolysis at some other free end which presents a maltose con-
figuration and hydrolysis will continue to the point at which branching
of this free end occurs with either the main chain or the branched chain,
as the case may be on which it is assumed that the gamma-amylose is
attached (granting there are no intervening blocks) and at which point
hydrolysis ceases.

If the radical is attached at some intermediate point (*e.g.* carbons
2, 3, or 6 of some intermediate dextrose group) then hydrolysis will
proceed from a free end (granting there are no intervening blocks) until
the glucopyranose unit to which the gamma-amylose radical is attached
is reached and further conversion will cease since a maltose configuration
no longer presents itself.

If it be argued in the last two examples that at the point at which
hydrolysis ceases the total unconverted residue is in reality gamma-
amylose, then some provision must be made to account for the soluble
dextrins that are produced during a barley diastase conversion. If it is
assumed that these dextrins originate from other starch molecules to
which no gamma-amylose radical is attached, then it must be admitted
that corn starch consists of at least two fundamentally different types of
molecules, the proof of which statement is the thesis of this paper.

Finally, if to avoid accepting the logical conclusions that follow from
postulating that gamma-amylose is but a radical attached to a larger
starch molecule, one prefers to believe that gamma-amylose is attached
to this molecule by a peculiar linkage, and that there exists in barley dia-
state an enzymatic component capable of splitting this linkage, then it
must follow that all starch molecules are not the same. The potato-
starch molecule must be constitutionally different from the corn-starch
molecule. Tapioca must, in addition, be different from either of these
two to account for its lower content of gamma-amylose. Otherwise we

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[3] Pringsheim, for example, proposed that the furanose form exists in starch, whereas the third
linkage in harmony with methylation data, 1-4, 5 acetal, has recently been considered by Hudson.

must assume that the molecular weight of tapioca starch is ten times as great as that of corn starch, a supposition which would find very little support. Hence, tapioca must contain some carbohydrate molecules like corn—with the gamma-amylose radical attached—and some without. If so much is granted, then the possibility cannot be denied that also within corn starch we might have radically different types of molecules. In this case the necessity for inventing the hypothetical linkage and the hypothetical enzyme to split it vanishes.

Furthermore, in conclusion, it would seem odd that cereal starches such as the corn-starch molecule should be supplied with a resistant barrier were it not to protect the reserve carbohydrate during its formation and storage in close proximity to the embryo. It would seem equally odd that at the same time the embryo, before germination, should be supplied with a special component capable of splitting this barrier from molecules of starch that happened to be exposed. We would rather believe that we have proved that gamma-amylose is a separate and distinct entity quite different from the major constituent, amylose, and that the former is located in the more accessible portions of the granule.

Experimental

Procedures for preparing corn gamma-amylose.—One kilo of commercial cornstarch is extracted with 80% dioxane, preferably in a reflux percolator, until the total fat content is 0.03% or less (according to the method of Sherman, 1932). The starch is quickly air-dried in a forced draft and washed at least three times by suspension in dilute alkali at pH 9. It is then suspended in a small amount of distilled water, passed though No. 20 silk, and made up to 10 liters. Sufficient alkali, either NaOH or KOH, is now added, in solution, to make the resulting paste 0.67 normal in respect to the alkali. It is stirred for 30 minutes at room temperature, whereupon the solution becomes clear and no insoluble starch-cell fragments are observable under the microscope. The solution is now neutralized with stirring for several hours until the paste reaches a pH of 6.2. It should not exceed 13 to 14 liters in volume.

To this paste an extract equivalent to 200 g. of finely ground barley is added, plus 100 cc. toluene. The barley is extracted with about 5 times its weight of cold water, in 2 extractions, the extract precipitated by adding an equal volume of ethyl alcohol and the precipitate redissolved in water and filtered clear.

After 24 hours' conversion at 55°C. the gamma-amylose flocks out and after cooling to room temperature it is centrifuged out and washed in the centrifuge to reduce the solubles. After dilution to approximately 5 or 6 liters and adjustment of pH to 6.2, it is again converted by a repe-

tition of the former treatment, this time with addition of but half the amount of barley diastase used at first.

After separation the second time, the gamma-amylose is washed free of reducing substances. It is then made up to a volume of one liter containing enough potassium hydroxide to make the normality 0.67 and stirred 30 minutes. It is centrifuged and the clear solution neutralized to pH 5.0 with HCl and allowed to stand overnight.

The precipitate is washed chloride-free in the centrifuge, dehydrated by successive washings with absolute ethyl alcohol, washed twice with dry ethyl ether, and dried in a vacuum desiccator.

Acetylation of gamma-amylose.—Ten grams of product was suspended in 62 g. of dry pyridine to which was added, drop by drop, 48 g. of acetic anhydride, with vigorous agitation. The reaction for the first 8 hours was maintained at 5°C. by immersion of the reaction flask in an ice bath. Thereafter for the next 64 hours the reaction was allowed to proceed at room temperature.

During the first few hours the mixture became very viscous and 100 g. more pyridine was added to keep the solution fluid, whereupon 48 g. more of acetic anhydride was added.

After 72 hours' reaction time solution was complete and the reaction was brought to 70°C. and held at that point under a reflux for one hour.

Cooled to room temperature, 250 cc. of $CHCl_3$ was added. The $CHCl_3$ mixture was washed with two 300-cc. portions of $4N$ HCl, then with two 250-cc. portions of dilute Na_2CO_3, and four times with water using 250 cc. each time. The product in $CHCl_3$ solution was then dried by the addition of the excess anhydrous Na_2CO_3, filtered clear, and evaporated to 120 cc. To this solution with vigorous agitation was now added, drop by drop, 150 cc. of petroleum ether. The precipitated mass was filtered dry by suction and washed with two 30-cc. portions of a 2:1 petroleum ether-$CHCl_3$ solution and then with petroleum ether. It was finally suspended in about 150 cc. petroleum ether, filtered and dried *in vacuo.* The yield was 8.5 grams. Found: acetyl groups, 44.1%, 44.5%; molecule weight in camphor, 4,890 ± 390.

Deacetylation of gamma-amylose acetate.—Six hundred and twenty-eight milligrams of the acetate was suspended in 6 cc. of normal NaOH at room temperature. After 30 minutes 5 cc. of H_2O was added and after 24 hours 5 cc. more of normal NaOH. After 48 hours, 25 cc. of water was added, at which point solution was practically complete. After 24 hours more, the solution was made up to 100-cc. volume in a closed volumetric flask and after 24 hours more, with occasional shaking, the optical rotation was determined at 25°C.

Determination of silica.—Gamma-amylose was analyzed for silica by first fusing it with sodium carbonate, leaching the ash first with water,

then with dilute HCl, combining the solubles obtained, and treating an aliquot by the procedures described by Schwartz (1934) and by Swank and Mellon (1934).

Conclusions

Methods for the isolation and characterization of an amylose fraction of corn starch have been presented, which product has been provisionally called gamma-amylose. Tapioca has been found to give but a tenth of the yield given by corn starch, whereas potato starch contains practically none.

Proof has been given that this amylose is different from beta-amylose or physical modifications of the latter, such as would be formed by reversion or retrogradation.

The molecular weight of gamma-amylose acetate is given which indicates that this amylose is a higher polymer of the units that make up beta-amylose.

Evidence has been submitted that gamma-amylose is not a product of synthesis from the intermediate degradation products of starch but exists preformed in the corn-starch granule.

Various theories used to support the modern concept of starch as consisting of but one fundamental type of carbohydrate have been criticized on the basis of the isolation of gamma-amylose and have been shown to be untenable.

The need for a more careful study of the multiple-amylose concept of starch before attempting to determine the configuration of a starch molecule has been pointed out.

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CONVENIENT MEASUREMENT OF GASSING POWER WITH DOUGH UNDER CONSTANT PRESSURE AND WITH ELIMINATION OF GAS SOLUTION

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Many devices for measuring the volume of gas liberated by a dough during fermentation have been described. Common imperfections in the devices have been absorption of the gas by water, varying pressures of the gas whose volume is measured, and inconveniences of manipulation.

In the apparatus of Jago and Kunis (cf. Elion, 1933) the pressure in the space above the water in the displacement vessel is reduced proportionately to the difference in level between the water surface
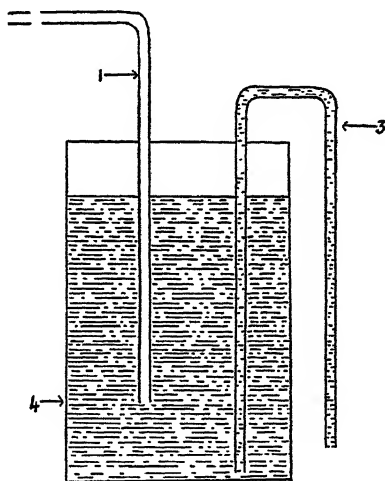
Fig. 1. Irvin's arrangement for constant gas pressure in the dough vessel.

in the vessel and the outer end of the syphon; and, further, the pressure in the dough vessel also is reduced, because the two vessels are in direct connexion. Irvin (1935) described an improved arrangement (Fig. 1), based on "Mariotte's bottle," in which the gas in the dough vessel remains at constant pressure. The inlet tube (1) from the vessel containing the dough is carried almost to the bottom of the water displacement vessel (4), instead of just into the top, as in the apparatus of Jago and Kunis. The length of the outlet tube (3) of the syphon is then adjusted so that with the inlet tube (1) open to the atmosphere, water just fails to drop from the syphon tube (3). This gets rid of

the variation in pressure in the dough chamber, as is shown in Irvin's manometric measurements. The pressure of the gas in the displacement chamber is reduced below atmospheric pressure by the head of water in the syphon—that is, the difference in level between the water level in the displacement chamber and the outer end of the syphon tube (3). The pressure in the dough vessel, however, is balanced at atmospheric pressure by the column of water between the surface of the water in the displacement vessel and the lower end of the inlet tube (1).

Any increase in pressure in the dough vessel causes a gas bubble to escape from the end of the inlet tube (1) up to the surface, where it causes a momentary increase in pressure until conditions are brought back to balance by the flow of water from the syphon. But as the water falls in the displacement chamber, the pressure of the gas in that chamber varies. Thus, the gas is produced under constant pressure but is measured under varying pressure, and Irvin fails to point this out in his description of "a constant pressure gasometer." It is true that the error is unimportant for routine measurements and that a correction can be readily calculated or determined for Irvin's apparatus, because here the corrections depend only upon the dimensions of the displacement vessel. With Jago's apparatus, however, account must also be taken of the errors due to the changes of pressure in the dough jar, and in the volume of the dough jar and its nongaseous contents. Irvin's apparatus also allows interruption of the experiment for dough punching and other manipulations, without the introduction of errors.

The fact that the gas pressure in the dough vessel remains at atmospheric pressure is a distinct advantage when measurements are required of the gas production of dough in large vessels or when the dough vessels vary in volume. Whatever the size of the dough vessel, a given quantity of gas will always displace the same volume of water from the displacement vessel, which would not be true if the gas in the dough vessel were not kept at atmospheric pressure. Irvin's apparatus is also useful if it is desired to keep the dough under normal pressure, as, for example, when measuring the gas production of dough pieces during tin proof. In this case, too, a relatively large container is required to hold the bread tin.

Irvin prevented the solution of the gas by using a salt solution but this does not allow the refilling of the displacement vessel from the thermostat in which it is immersed.

The devices described below possess all the advantages of Irvin's design but, in addition, avoid the use of a salt solution and also allow rapid filling of the apparatus from the thermostat.

Fruit-preserving jars, with metal screw tops and fitted with brass tubes, are very suitable, but either apparatus can be made of metal if desired.

One method is illustrated in Figure 2. Inlet and outlet tubes (1) and (3) correspond exactly to the corresponding ones in Irvin's apparatus but a wide tube (2), open at both ends, surrounds the inlet tube (1). At the bottom of the vessel is a layer of heavy liquid (for example, nitrobenzene or diethyl phthalate) which is not miscible with water and in which the gas is insoluble. When the apparatus is first
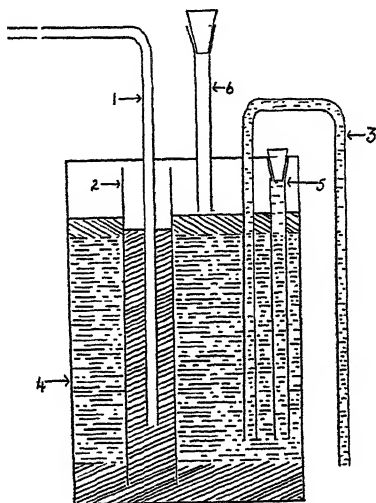
Fig. 2. Arrangement permitting filling of the apparatus from the thermostat, by avoidance of salt solutions.

set up and before any water has been run in, the heavy liquid is introduced. As water is now run in, the heavy liquid rises in tube 2 to a height depending on the water level. It will be seen that the incoming gas will now pass, not through water in which it would dissolve, but through the heavy liquid in tube 2. The end of tube 1 will be under the same static pressure as it would be if water only were used, because since tube 2 is open at both ends, a condition of equilibrium establishes itself. A layer of oil on the surface of the water prevents solution of the gas there.

Refilling takes place through tube 5, the upper end of which is flush with the top of the vessel and opens into the thermostat and the lower end is just clear of the heavy-liquid layer. The water in the thermostat covers the top of tube 5. During refilling, gas and air escape through tube 6, which extends a short distance into the vessel.

When the water rises to the level of the bottom of tube 6, air or gas can no longer escape, so that the water is prevented from rising high enough to flow down tube 2.   Tubes 5 and 6 are closed by stoppers during measurements.

The inner leg of the syphon tube (3) ends clear of the heavy-liquid layer, so that there is no danger of this liquid escaping.   A dye, Sudan Red, for example, is dissolved in the oil, so as to indicate immediately if the measuring capacity of the vessel is being exceeded.

Both tubes 1 and 6 should extend about four inches above the surface of the water in the thermostat.

The outer leg of the syphon tube (3) is made longer than finally required, and then its correct length determined as follows.
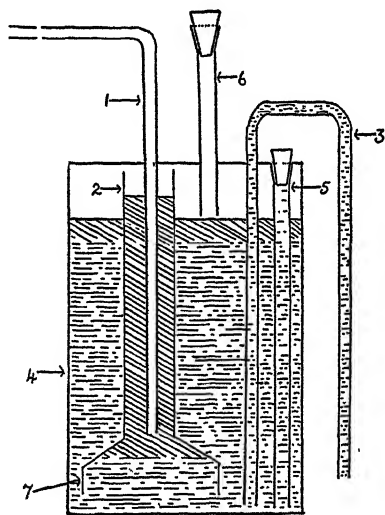
Fig. 3.   Alternative arrangement permitting the avoidance of salt solutions.

Fill the vessel by opening tubes 5 and 6; fit a few inches of rubber tubing on to the outlet of the syphon; close 5 and 6 and, with 1 open, start the syphon by gentle suction on the rubber tube.   Allow the syphon to run for a short time and then slowly raise the free end of the rubber tube until the flow just ceases.   Cut the syphon tube at this level.   Minor adjustments can be made by bending the tube so that a drop of water just fails to fall.   In practice this is a simple adjustment.

All the manipulations so far described are done once for all when the apparatus is first set up, and they never have to be repeated during routine work.

Now, if we are to investigate so worthy a subject, may we receive the immortal and indeed divine motivation, by which alone we are, and indeed by which alone we are nourished? Very rightly, the Greeks, the Romans, the Arabs and all those who *summa cum lauda* are following them, the Gauls and the Italians put forth their efforts to determine and explain the nature of foods. Their worthy studies stand out so that we may well admire their excellent observations—indeed we may wonder what they did not observe. It is splendid indeed to investigate the matter of foods and to derive hope from the extent and the almost infinite variety of such materials.

In realizing this, Beccari did not hesitate to apply his diligence to foods which may be of the greatest value to a community and of which all are partakers. Thus faced, he discovered two species of substances in wheat flour, strangely different in themselves, which can be separated with ease, and be discerned as individuals; on the one hand, one may disclose the similarity of these things which are extractable from vegetable bodies, but in this there would be nothing worthy of admiration; on the other hand one may seek and compare, as is possible with those materials removed from the bodies of any living creature; this Beccari reported very admirably; it may well be wondered as to how other authors failed to record similar observations, but nowhere is this evident. He communicated this study to the academy in 1728 in a long lecture to which detailed reference will be made.

First, however, the two parts of the flour to which reference has been made will be explained. They may be obtained with little difficulty. Flour is obtained from the best wheat, moderately ground so that the bran will not pass through the sieve; from this it follows, therefore, that the product is of the cleanest with impurities removed. This is mixed with the purest water, and is kneaded. The residue obtained in this operation is accomplished by washing. The water, therefore, carries away all the portions that can be dissolved; the other portions it leaves behind intact.

Moreover, those portions which the water leaves behind, manipulated by hand and pressed under the residual water, are little by little gathered in a soft and—as much as possible—a tough mass; an excellent kind of paste, and most suitable for the work that is to be done; it is most noteworthy how it does not permit itself to be mixed with water. Those other portions, which the water carries along with itself, remain suspended a considerable time and render the water milky; afterwards they settle downward little by little and collect at the bottom, yet they do not cohere very much with each other; at the slightest disturbance they again float upwards like dust, as it were. Nothing is similar to this starch (" amylo "); this food is truly amylaceous. But it is in fact of two

kinds of parts as Beccari proposed on the basis of his chemical work, and to which he applied names to distinguish them, calling the one glue-like ("glutinosum") and the other starch-like ("amylaceum").

So great is the difference of these two fractions that if subjected to resolution by digestion, or by distillation, one obtains principles from each that are quite different; the starchy material yields principles which disclose its vegetable origin, whereas the gluten when thus treated behaves as though of animal origin. So that this may be understood better, it may be convenient to know in general just how they exhibit diverse characteristics which are as of vegetable and animal nature, and are disclosed in digestion, and in distillation.

In digestion, which is effected by low heat of long duration, the parts of any animal are never brought to a real and absolute fermentation, but they are always caused to putrefy with a very bad odor. The vegetable parts may be fermented naturally, as it were, and not caused to putrefy unless this is brought about artificially; they yield certain characteristics of sourness during fermentation which are in no way like the behavior of animal parts when they are caused to putrefy. Also, after long continued fermentation, the vegetable portions may produce an acetic liquor which has a savour of wine; the animal portions, when caused to putrefy, yield a urine-like liquid. Out of one a strong spirit can always be recovered, and appreciable acid salt; out of the other (animal) there is no trace of acid salt but not a little alkali. Finally, the vegetable portions, when they have been fermented, relinquish a substantial amount of *tartar* and of stable salt; of which salt none appears when animals are caused to putrefy. These are the differences between vegetable parts and the parts of animals in digestion.

Whereas the fresh and sound parts of animals and plants may be so distilled, they differ also when dried over a strong fire. From the animal parts water is first extracted, entirely devoid of taste; then a vapor becoming yellow and also alkaline; abundant salt follows this, equally alkaline, volatile, solid, and blended in a yellow oil, indeed beautifully golden and rank; if the salts become separated from the oil by the heat, they become bright like snow. In an extremely increased fire another heavy oil appears, dark, viscous, with a very bad odor, adhering to the bottom of the vessel, sponge-like, flat in taste, dark, and unlike anything above mentioned; later this can be whitened on the open fire as has been shown. However much one washes this out, one may extract no salt. Such is the distillation of animal parts. The vegetable parts, however, produce first a watery liquid, very acid, which becomes more acid over an increased fire, and also more reddish; meanwhile a light and yellow oil comes out; finally, at extreme heat the vapor becomes very acid and thick; likewise the heavy, thick and dark oil floats like a fish.

In this way the plant and animal parts differ, as to chemical considerations, as we have summarized; so they are seen to produce alkaline principles elicited from animal parts, and acid principles from vegetable parts.

After this discussion concerning them, to which we have digressed, we again return to the parts of wheat flour, starch and gluten; as they emerge from any one substance they are discernible as such, and they may be had for demonstration either in digestion or in distillation; for starch, as I have said above, appears to be entirely of vegetable nature; gluten, however, exhibits the characteristics of animal parts, so that whoever may not know it to have been taken from grain might assert without the slightest doubt that it came from animal material.

We may begin with digestion. This glutenous material produces no characteristics of sourness; it smells bad within a few days and spoils; it putrefies strikingly like a dead body; it rots, it may become fluid, and it may then be dispersed in water, leaving very little blackish material in the container; it produces quickly a mass like a spoiled carcass. Starch, however, does not initially yield an acid, then sours perceptibly, and finally contracts the odor and flavor of wine and becomes blended with the supernatant liquid. The first (gluten) is characterized by its tendency to rot, the latter (starch) by becoming sour; surely Beccari might have obtained this less in a temperate place, since, for instance, in the more than forty day period during the warmest time of spring spoilage would have occurred more easily, nevertheless he preserved the odor and somewhat acid taste, always fresh and unspoiled.

He preferred to conduct his experiments in water, in which the digestion of either the glutenous or the starchy part may be achieved. In the instance of the starchy part, all comes to the same state; indeed, the water comports itself as though it was accustomed to blending with the acids that are produced. Nothing is changed during the mixing of the other acids; with the alkaline salts, as for instance with the tartar salts, it effervesced slightly indeed but sufficient to foam over, and, furthermore, lost its clearness. Then it threw off whitish particles, followed by a violet color which merged into a red color that was retained.

The water which was affected by the glutenous portion, however, acted in a manner as though the alkaline juices are accustomed to it. It effervesced, due to the mixing of its acid; it changed in color, and, after a few days it settled to the bottom as a very fine powder.

Beccari was unwilling to remain silent concerning these particulars, which have been known with certainty in these color changes which are mentioned above. These he narrated, therefore. The liquor extracted an unfading color out of the water in consequence of strong mixing, of beautiful appearance which becomes visible after more or less saturation.

The liquor becomes a reddish color, with a constant acetic content. If Ettmullerus had actually recognized this when he tried to discover the change in color in chyle, the experiments of Fluddius might not have been made. Fluddius elicited such a substance out of bread, which was very white, but when carefully closed up in a transparent vessel and placed in the sunlight, developed little by little either a purple or red color much as does chyle which may be very white and change into blood-red. As Beccari assumed, the change of color which he observed may be attributed to the same phenomenon.

We now return to the proposed " liquid." When disturbed by added water which has imbibed mercuric sublimate it becomes turbid; with water which has imbibed salt of lead it is no less turbid; with alkali it does likewise. Moreover, it deposited a very fine and white powder at the bottom which, most certainly, is produced by the volatile alkali. Thus both fluids from the respective portions from which they were obtained, may be clearly divided naturally; further, so far as may be manifested by the distillate, the moisture which is expressed by distillation out of the latter (starch) had all the characteristics of an acid nature, and that out of the gluten, the characteristics of an alkaline nature.

While these characteristics may be discovered by digestion of these two portions of wheat flour, that is, the gluten and the starch, the distillation did not disagree with the digestion. Also when these materials, heretofore thoroughly discussed, are not brought to a state of decay by digestion, they may be experimented with by distillation; the gluten portion which yields little or a small amount of water, after a little gives off a vapor similar to urine, followed by an oily material, and then some rather strong, volatile salt such as is given off by such treatment of a stag's horn. The starchy part indeed, yielded nothing at first except a little water, then much vapor of acid, and last of all not a little oil; always, however, it yielded characteristics of an acid nature which attest to its vegetable origin.

Thus not only the chemical digestion but also the distillation has shown the two natures, and these in themselves very different, in one and the same flour. The investigation of this thing allured Beccari. For this reason he desired to prove the same also in flour from beans, from barley, and other things. But there was no like response except in the case of spelt. With the other things the water rinsed everything away so that no glutenous or other compact material remained behind which could be compared with the gluten of wheat; so that Beccari marveled at so great an unlikeness in similar things. All of these flours, as in the instance of the starchy material to which reference has been made, when diluted with water, and properly heated at the right temperature, came together in a cohesive mass, so that they may be used

as a glue on paper; but this has nothing to exhibit in common with the paste of flour. The starch of wheat, if and when opportune, acquired a firmness, and this firmness alone is better than in all flours; also of barley, which attracted the first admiration of Beccari, so that he formerly supposed this flour to be the most cohesive of all. He confirmed this experimental opinion. The old investigators had observed this and maintained that foods made from barley would give acute trouble to hard workers, and bread which is made from wheat they called the best of all. However this may have been, it certainly is evident that Beccari discovered this advantageous unlikeness in many kinds of flours, and, what is still greater, the differences in the constituents of wheat flour alone; these, however, are less significant if we recognize how varied and multiple are the interrelations of all things. However, it was the custom of the scientists of the past that they strived to obtain a likeness and a constancy, but due to their nature, they may have been misled as to the variability. Also it is not apparent that this variability may not have been more beautiful than the perpetuity which they sought.

This statement concerning grain and flour has been presented so that we may interest others, if possible, to follow Beccari in the study of the materials in foods; it is indeed a study worthy of a scientist in which the matter of being ill as well as of being healthy may be investigated.

BIOLOGICAL ELIMINATION OF GLUTATHIONE
FROM WHEAT GERM AND FLOURS
USED IN BREAD MAKING

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The

a fact t; injurious effect of wheat germ on dough and loaf properties is for this that has been established for a long time. Different explanations 1924; '; phenomenon have been brought forward. Lipoids (Working, (Cairns Sullivan and Near, 1928; Geddes and Larmour, 1933), proteases sen, 19; and Bailey, 1928; Balls and Hale, 1936a, 1936b, 1938; Jørgen-Walde and 1939; Flohil, 1936; Hale, 1939), fatty acids (Swanson, 1934; 1938), and re ngels, 1930; Kosmin, 1934a, 1934b, 1935; Barton-Wright, sen, 1936; Sull iced glutathione (Balls and Hale, 1936a, 1936b; Jørgen-1938) have in t an, Howe, and Schmalz, 1936, 1937; Ford and Maiden, and germ-contai rn been made responsible for the harm done by germ ing mill streams to bread texture. The last-mentioned

explanation appears to be the one that is best supported by facts. It seems, moreover, to account for the favorable effect of potassium bromate and other oxidizing bread improvers, and for the improvement of flour by heat.

In the course of a study of the effects observed in ripening doughs, one of us (E. W. H.) set experiments to see whether the " greening " effect of the germ could be eliminated by a process of prefermentation with yeast. It was found that when a ferment consisting of wheat germ, water, and yeast was allowed to ferment for a number of hours and was then made into a dough, the bread made therefrom no longer showed the harmful effects of germ on baking quality. Later it was found that at the point at which good bread could be made from the ferment, reduced glutathione could no longer be detected with the sodium nitroprusside reaction.

From these observations a commercial process was devised which allows the addition to bread of up to 10% of wheat germ (on a flour basis). There has already been published (Hullett, 1940) a brief note on this process, in which was described also the improvement resulting from prefermenting the low-grade portions of very high extraction flours and in which it was suggested that glutathione elimination by yeast might be a part of the ordinary dough ripening process.

The experiments and findings made in working out the process outlined above are thought to be of sufficient general interest to justify their being published, although they raise problems rather than solve them.

The flour and germ used in the experiments were supplied by various New Zealand mills and were milled from New Zealand and Australian wheat. Throughout the present investigation the germ was used in uncomminuted state. The presence of glutathione in the germ was checked by means of the sodium nitroprusside reaction. For the test a sample of the germ suspension or paste was diluted to a proportion of 1 part germ to 30 parts water and strained through bolting sile. The glutathione used was a product from Hoffman-La Roche, B?

Effects of Glutathione and of Germ on Dough

The results obtained by earlier investigators are fully confirmed by the the farinograms and the results of the baking tests carried out ons was authors. It was found that the injurious effect of germ additigensen's greater with some flours than with others. According to the flours. theory this would imply variation in the protease content rmented germ

Chemical changes due to an addition of raw or of fecrease of cold to the dough seem mainly to consist in an increase or c water extract, which can be seen from Table I.

TABLE I

COLD WATER EXTRACT

Additions on flour basis: 2% yeast; 2% salt; 1% cane sugar; 4% raw germ or 12% moist germ ferment equal to 4% germ. $4\frac{1}{4}$ hours' fermentation at 82°F. Two knocks after 2 and 3 hours.

	Fresh dough	Fermented dough	Bread
	%	%	%
No germ	4.9	4.2	5.0
Raw germ	5.5	5.0	5.4
Fermented germ	5.0	5.0	4.9

Considering that a content of 5% cold water extract in the dough means that the liquid phase surrounding the gluten strands has a concentration of roughly 11%, it is possible that changes in its composition might affect the gluten properties, and from this viewpoint the increase in cold water extract caused by the addition of raw germ should be noted (cf. Kosmin, 1933). But the evidence is insufficient to explain the effect of germ in terms of chemical composition. More detailed chemical analyses were carried out but up to the present no clear conclusion has been reached.

Prefermentation of Germ and Dough Properties

Table II gives observations on a ferment sufficient for a sack of flour (200 lbs). Enough of the ferment to make one loaf was taken at the sixth and following hours, and doughed up at 73°F. Each dough was allowed to lie for half an hour, rounded up, left for half an hour, and then tinned up. All the loaves were satisfactory from the seventh hour onwards. The crumb color varied from a light brown in the sixth hour to a pinkish brown in the 11th hour. This is discussed later.

TABLE II

PREFERMENTATION CONDITIONS SUITABLE FOR 200 LBS. FLOUR

Formula of ferment: germ, 20 lbs.; yeast, 1 lb. 7 ozs.; water, $3\frac{1}{2}$ gals. The " gassing power" is the cc. of gas given off by 78 g. of the main ferment enclosed in a jar embedded in the main ferment so as to experience the same temperature changes.

	Hours								
	1	2	3	4	5	6	7	8	9
Gassing power for each hour (cc.)	95	150	173	216	186	120	81	35	20
Ferment temperature (degrees F.)	74	74	77	$80\frac{1}{2}$	82	82	80	78	76
Glutathione test: + = present − = absent	+	+	+	+	+	slight	−	−	−

It was apparent that once the glutathione had disappeared, all the loaves were satisfactory, which shows that, if necessary, a baker could leave the ferment beyond the time it is ready and still get good bread. It is a striking feature of the addition of fermented germ to bread that it has a maturing effect and gives good tolerance to the dough. Doughs containing 10% (on flour basis) of fermented germ were found to yield fully satisfactory loaves after two hours' fermentation (from mixer to oven) in the dough, and to continue to give equally satisfactory ones over a period of six hours. This at least applies to New Zealand flours; it may be different with other types of flours.

The following examples show how the process is carried out commercially:

(a) 20 lbs. of germ, 6 lbs. of yeast, and 4 gallons of water are made into a ferment, which is kept at 80°F. for 4 hours. 200 lbs. of flour, 4 lbs. of salt, any other dough ingredients, and the required water are mixed to a dough, from which loaves can be taken off starting from 2 hours after mixing.

(b) 20 lbs. of germ, 1½ lbs. of yeast, 4 gallons of water, fermented at 74°F. for 9 hours. Dough making as under (a).

As has been mentioned above, the practical use of the process is not limited to wheat germ additions only. It has been found that the principle of prefermentation can successfully be applied in improving the baking quality of low-grade flours. A very satisfactory loaf is obtained when low-grade flour (the fraction 70%–80% on wheat basis) is prefermented and straight flour (0%–70% extraction fraction) is added in dough making. The photographs (Fig. 1) show a normal bread loaf,

Normal loaf   10% fermented germ   10% untreated germ

Fig. 1. Effect of prefermentation with 10% of wheat germ in the flour.

one containing 10% (on flour basis) of prefermented germ, and one containing 10% of untreated germ.

If the branny part of a whole meal is prefermented separatedly, and the remainder added when dough is made, an improvement over an ordinary straight whole-meal dough is obtained with wheats of poor baking

performance. Rat-feeding trials [1] showed that the vitamin B_1 of the wheat germ was not destroyed by the process.

It might be argued that the maturing effect is due to yeast multiplication taking place during the fermentation of the wheat germ. In order to make sure whether this was the case, yeast cells in fermented and unfermented germ ferments were counted, with the following result:

20 g. germ + 40 cc. suspension of 10 g. yeast:
    (a) kept in refrigerator for 4 hours—1,040,000 cells per mm.[3]
    (b) kept at 82°F. for 4 hours—1,120,000 cells per mm.[3]

The yeast cells were roughly separated from the ferment by the method which will be described later under the heading " Effect of Germ Fermentation on the Yeast." The difference of 80,000 cells per mm.[3] falls within the margin of error of the counting method. Hence the maturing effect is not due to yeast multiplication.

Characteristics of Germ Fermentation

The fermentation which leads to the destruction of glutathione in the germ is connected with an enzyme mechanism effective in the raw germ. This is evidenced by the following:

1. Glutathione is not destroyed by boiling of germ, and when boiled germ is fermented with yeast, there is no destruction of glutathione as there is with raw germ. The boiling evidently destroys the enzyme.

2. Fermented raw germ contains no glutathione SH but may be supposed still to contain the enzyme and therefore to have the power of bringing about the elimination of the glutathione present in a ferment of boiled germ. This does in fact take place.

3. Pure glutathione was added to (a) a fermenting sugar solution, (b) a ferment of boiled germ, and (c) a ferment of unboiled germ. Only in the last case was the glutathione destroyed.

Study of the Rate of Glutathione Destruction in Fermenting Wheat Germ

Some indication has already been given of the speed of the process. It was found that the time taken for the destruction of glutathione could be considerably shortened by increasing the yeast quantity and lengthened by decreasing it. For instance, the time required for glutathione destruction with the same initial conditions as given in Table II but with four times the yeast quantity was 3 hours to 4 hours according to the glutathione content of the germ.

With commercial formulas employing relatively high concentrations of the germ and yeast, temperature variations are very difficult to avoid.

It was therefore realized that a more dilute system was better suited to a study of the effects of variations in the concentrations of the different constituents. Tables III, IV, and V record observations on relatively dilute ferments.

TABLE III

EFFECT OF GERM AND YEAST CONCENTRATION ON GLUTATHIONE DESTRUCTION IN GERM SUSPENSIONS CONTAINING 75 CC. OF WATER, FERMENTED AT 82°F.

Wheat germ	Yeast	SH reaction
g.	g.	
2.5	1.0	− after 5 hrs.
2.5	2.5	− " 4½ "
2.5	5.0	− " 4–4½ "
5.0	1.0	− after 6½ hrs.
5.0	2.5	− " 5½ "
5.0	5.0	− " 5 "
10.0	1.0	+ after 6½ hrs.
10.0	2.5	± " 6½ "
10.0	5.0	± " 6½ "

− means negative; + weak positive; ± almost negative

TABLE IV

EFFECT OF pH ON GLUTATHIONE DESTRUCTION IN GERM SUSPENSIONS FERMENTING AT 82°F.
20 g. germ + 10 g. yeast in 200 cc. diluted HCl. Yeast: liquid

Initial pH	Glutathione reaction after 5 hours
4.45	+
4.72	±
5.12	±
5.58	±
6.10	±
6.41	+

TABLE V

EFFECT OF TEMPERATURE ON GLUTATHIONE DESTRUCTION IN GERM SUSPENSIONS FERMENTING AT DIFFERENT TEMPERATURES
20 g. germ + 10 g. yeast in 100 cc. water

Initial temperature	Time required for glutathione to disappear
82°F.	4 hrs.
94°F.	3 "

The rate of glutathione destruction in fermenting unboiled germ can be supposed to depend on the following factors: concentration of glutathione, concentration of the active enzyme, and probably rate of fermentation. Temperature and pH, besides affecting the rate of fermen-

tation, might have a specific effect on the enzyme. It was not possible to study the effect of separately varying the glutathione and enzyme concentration, as these two are associated in the germ and no adequate supply of pure glutathione was available.

Glutathione destruction was slightly accelerated by using a $0.002N$ solution of sodium cyanide instead of water. When a $0.001N$ solution of iodoacetic acid was used instead of water, gas production as well as glutathione destruction was entirely stopped. Yeast treated with acetone failed to produce fermentation and no glutathione destruction took place. Neither did glutathione disappear when sodium fluoride was applied to such an extent as to stop fermentation. Thus it was not found possible to disconnect the destruction of glutathione from fermentation.

The conclusions from these trials can be summarized as follows: Increase of temperature (up to about 35°C.) and yeast quantity and decrease of initial pH to 5–5.5 shortened the time required for the SH reaction to become negative. The figures in Table III appear, besides, to indicate that decrease in germ concentration accelerates glutathione destruction. But the converse is found with the tight ferments used commercially. The impression was received that this is due to opposing effects of glutathione and enzyme concentration in the germ.

A series of gassing tests was run in order to find out whether the fermentation of raw germ under different conditions exhibits, in the gassing rates and total gas production, any characteristics differentiating it from the fermentation of boiled germ suspensions or of sugar solutions of comparable sugar and yeast concentrations. The use of a $0.001N$ solution of iodoacetic acid instead of water in dough making produces slackening and stickiness of the dough. Farinograms obtained from such doughs indicate a strong degrading effect taking place.

In order to obviate sugar production by amylases acting on any starch in the germ samples the experiments were carried out on centrifuged extract. The germ used contained 0.5% reducing sugar (expressed as invert sugar) and 14.7% of sucrose, making a total of 15.2%. The sugar content of the sugar solutions without germ was adjusted accordingly. ~

Germ extracts of two concentrations and solutions with corresponding sugar concentrations (1.5% and 1%) were fermented with an addition of 5 g. of yeast per 100 cc. The initial gassing rate of the germ extracts was higher, no doubt owing to their high protein content and the presence of vitamin B_1 (Schultz, Atkin, and Frey, 1937). The totals for four hours' fermentation and the shape of the graphs of gassing rates give, however, no indication that the fermentation going on in fermenting raw germ is of a type different from that taking place in fermenting boiled germ or in fermenting sugar solution.

The decrease of pH taking place during fermentation was also practically the same with raw and boiled germ, *i.e.*, from about 6.7 to about 5.5.

Color Formation in Fermented Germ

In all our experiments, whatever concentration of germ or yeast was applied, the surface of the fermented suspension or paste began to turn dark brown, later violet, as soon as the glutathione had disappeared and this color deepened as time went on. The bread likewise acquires a color varying from almost white to a pinkish brown, depending on the period of fermentation after glutathione has disappeared. This process seems to be due to oxidation of carotene by air. This is concluded from the fact that extraction of carotene from the germ by means of petroleum ether prevents the color from developing. Low temperature fails to prevent the formation of the color on the surface of fermented germ suspensions, but absence of air or substitution of air by CO_2 does prevent it. Addition of sugar to the ferment also retards the development of the color, and this is no doubt due to sustained CO_2 production.

When the initial pH of the germ suspension is adjusted to values from 5.5 downwards a very noticeable repression of color formation takes place. Bread baked with an addition of acidified germ ferment (equalling 10% germ on flour basis) was of almost normal color. The pH adjustment was made by means of HCl (1 cc. $0.10N$ HCl for every gram of germ).

No color formation occurs in boiled ferments, which suggests that an enzyme mechanism is involved in this process also.

Fate of Glutathione in Germ Fermentation

It has been suggested by different authors that glutathione undergoes oxidation when germ is allowed to age or is heated under certain conditions. These authors also attributed the maturing effect of $KBrO_3$ to glutathione oxidation. Starting from this hypothesis we originally anticipated that the disappearance of the glutathione reaction in the course of fermentation was caused by oxidation to the SS form. In order to test this hypothesis attempts were made to reduce the supposedly oxidized glutathione. Reduction was attempted by: (*a*) saturating the solution with H_2S in presence of a few drops of mercury; (*b*) using aluminum amalgam in neutral solution; (*c*) using zinc and sulfuric acid; and (*d*) using sodium cyanide solution. None of these methods yielded the least trace of reduced glutathione.

In order to make sure that the reduction of the S–S form to the SH form could indeed be effected by these methods, pure glutathione (SH)

was oxidized according to Hopkins (1929) and the solution treated with zinc and sulfuric acid. A strong reaction with sodium nitroprusside was obtained after two hours, and this increased with longer reduction. (In the course of these experiments it was noticed that glutathione is easily adsorbed by various precipitates. In using aluminum amalgam, for instance, the glutathione SH is fixed to the aluminum hydroxide formed in the course of the reaction. The use of zinc has the advantage that the red color obtained with sodium nitroprusside and ammonia is much more stable in the presence of zinc hydroxide than otherwise.) The precipitate formed by the addition of sulfosalicylic acid (used by Sullivan, Howe, and Schmalz, 1937) was also found to adsorb glutathione.

As it seemed possible that the SH reaction of a germ extract might be masked by substances occurring in the extract, the following experiment was made in order to test this point: 0.025 g. of pure reduced glutathione, dissolved in 15 cc. water, was oxidized by adjusting the pH to 7.6 with $Ba(OH)_2$ and aerating until the sodium nitroprusside reaction became negative (Hopkins, 1929). This oxidation required six hours. The solution of the oxidized form was added to 30 g. of germ which had been fermented until the SH reaction had become negative. After thorough mixing, the suspension was centrifuged and the liquid treated with zinc and sulfuric acid. On standing overnight the solution gave a very strong SH reaction, which demonstrated that the nitroprusside reaction is not interfered with by any constituent of the fermented germ. It was likewise found impossible to obtain a positive SH reaction by reducing an extract from germ which, after having been kept in a closed container for several months, did not react any more with sodium nitroprusside. It can be inferred from this evidence that in fermenting or in aging germ the glutathione is destroyed rather than oxidized.

On the strength of the above-discussed findings it may seem doubtful whether the action of potassium bromate in a dough is indeed an oxidizing one. The view that $KBrO_3$ does not cause glutathione oxidation, at least not under the conditions prevailing in baking, is further supported by the results published by Ziegler (1940), who found that below 40°C. the oxidation of glutathione by $KBrO_3$, particularly in a CO_2-saturated solution, proceeds very slowly.

The practical aim of the authors' experiments did not justify an extensive investigation of what actually happens to the glutathione under the conditions discussed above. So much only was ascertained, that the destruction is not connected with H_2S development, as no blackening of lead acetate by the vapors coming from fermented germ could be detected.

Effect of Germ Fermentation on the Yeast

Attempts to find out whether the disappearance of glutathione from the germ extract corresponds to an increase of glutathione in the yeast cells led to the discovery that, on the contrary, the glutathione content of the yeast is greatly diminished. This is shown by the following experiments, in which a comparison was made of the glutathione contents of (a) yeast which had been fermented in a raw germ suspension, (b) yeast which had been mixed with a raw germ suspension which was kept in the refrigerator, and (c) yeast which had fermented a sugar solution of equal sugar concentration.

(a) 20 g. germ plus a 40 cc. suspension of 10 g. yeast in water, fermented at 32.2°C. for 3½ hours—SH reaction of fermented germ was negative.

(b) 20 g. germ plus a 40 cc. suspension of 10 g. yeast, kept in the refrigerator for 3½ hours—SH reaction positive.

(c) 3 g. sugar plus a 40 cc. suspension of 10 g. yeast, fermented at 32.2°C. for 3½ hours—SH reaction negative.

From each of these ferments the yeast was separated in the following manner: The ferments were diluted and strained through a fine bolting silk and the residues washed until the washings were practically free of yeast. The filtrates were then centrifuged until microscopic examination showed that negligible numbers of yeast cells remained in the supernatant liquid. The yeasts thrown down were each mixed with 20 g. of sodium chloride and allowed to stand overnight in order to bring about autolysis of the yeast cells. The autolysates of (a) and (b) contained, in addition to the autolyzed yeast, some starch and coagulated protein, which would not interfere with the tests. Each of the autolysates was made up to 100 cc. with water and again centrifuged. The liquids so obtained were tested for glutathione SH with sodium nitroprusside. The yeast from the fermented germ suspension (a) gave an almost negative reaction, while those from the unfermented germ suspension (b) and from the fermented sugar solution (c) gave strong positive reactions.

On the assumption that the glutathione originally present in the yeast of the autolysate (a) had been oxidized, attempts were made at reduction back to glutathione SH. These attempts failed, showing that the glutathione in the yeast, as well as that in the germ, suffers some more far-reaching change than oxidation to the S–S form.

Summary

It is shown that glutathione is eliminated from raw germ by fermentation.

The effects of germ, raw and fermented, on the properties of dough and bread are shown.

The connection between the presence of glutathione in germ and dough deterioration by germ addition is confirmed.

It is shown that prefermented germ or extracts from prefermented germ have a marked maturing action on doughs.

Evidence is given that an enzyme mechanism is involved in the elimination by fermentation of glutathione from raw germ.

The disappearance of glutathione from fermented germ is not due to oxidation to the S–S form; neither is this the case in aged germ.

The fermentation occurring in fermenting germ suspensions is shown to be an ordinary alcoholic fermentation.

It is shown that when raw germ is fermented, not only the glutathione in the germ but also that in the yeast is eliminated.

The application of the principle of fermenting germ to the making of a germ bread is reported.

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THE DETERMINATION OF FURFURAL-YIELDING SUBSTANCES AND FERMENTABLE CARBO-HYDRATES IN GRAIN [1]

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Knowledge of the content of fermentable substances in grain is of prime importance to those industries that ferment grain to alcohol. The research reported here was undertaken in order to establish a means of arriving at the true fermentable content of starch in grain. The acid hydrolysis method for starch was used to determine both fermentable and unfermentable carbohydrates. The rapid bromine method was modified and applied to the determination of the unfermentable carbohydrates, the so-called "pentosans" in grain. The true fermentable substance was obtained by subtracting the pentosan content from the "starch" determination.

time of reaction would be exact. Occasionally the reaction mixture was shaken. At the end of 4 minutes, 10 ml. of 10% potassium iodide, also at 0°C., was pipetted into the reaction mixture. The flask was shaken well with particular care that no fumes escaped. The contents were titrated with 0.1N sodium thiosulfate with starch indicator added at the end of the reaction. A blank was run on 100 ml. of 12% hydrochloric acid in exactly the same manner. The number of milliliters of thiosulfate used for the sample was subtracted from the blank.

Calculation: 1 molecule of thiosulfate was equivalent to 0.0082 g. of pentosan. Number of ml. of 0.1N sodium thiosulfate × 3 × 0.0082 = grams pentosan present in sample.

$$\frac{\text{Grams pentosan} \times 100}{\text{Weight of sample}} = \frac{\% \text{ pentosans}}{(\text{wet basis})}$$

$$\frac{\% \text{ pentosans (wet basis)} \times 100}{100.00 - \% \text{ moisture}} = \frac{\% \text{ pentosans}}{(\text{dry basis})}$$

In order to be satisfactory for general use, the results must check with those obtained by a standard determination. In this case the standard is the phloroglucinol method. Accordingly, pentosan determinations were run on samples of rye, corn, barley malt, and distiller's dried grain by both methods. The results checked very well as shown in Table IV.

TABLE IV
PERCENT PENTOSAN IN GRAIN

Sample	Mois-ture	Bromate-bromide method					Phloro-glucinol method		Dif-fer-ence, dry basis
		Wet basis		Dry basis		Aver-age, dry basis	Wet basis	Dry basis	
		I	II	I	II				
	%	%	%	%	%	%	%	%	%
Rye	8.74	8.78	8.79	9.62	9.63	9.63	8.75	9.59	0.04
Barley malt.... ...	8.51	10.53	10.58	11.51	11.57	11.54	10.57	11.55	0.01
Corn [1].............	10.10	5.28	5.37	5.87	5.97	5.92	5.39	5.99	0.07
Distiller's dried grain	8.31	13.98	14.17	15.24	15.40	15.32	13.90	15.16	0.16

[1] Same as sample 4 in Table II.

⌐ In the volumetric method, distillation was stopped at 270 ml., since a test for furfural in the distillate from the various grains after this amount had been collected proved negative. The test for furfural was conducted as follows: 1 ml. of a mixture of equal portions of aniline, glacial acetic acid, and water gave a characteristic pink test in the distillate prior to the collection of 270 ml. and no color reaction in 10 ml. of the distillate collected after 270 ml. Interfering nitrates were tested

for in the distilling mixture and distillate at intervals of 10 minutes throughout a distillation for each type of grain, and in every case the test showed negative.

The final experiments were carried out to show the true fermentable substances in corn and rye. The starch content of the same six samples of corn and rye was run according to the acid hydrolysis method of the *Official and Tentative Methods of the Association of Official Agricultural Chemists*. The pentosan contents were subtracted from the found starch contents to give a clearer picture of the fermentable substances in the grain. The data are listed in Table V.

TABLE V

Starch and Pentosan Contents of Rye and Corn Samples Showing the True Fermentable Substances Present, by Difference

Sample	Starch, dry basis	Pentosan (average), dry basis	True fermentable substances, dry basis
	%	%	%
Rye			
Eastern North Dakota	65.42	11.00	54.42
Eastern South Dakota	65.87	11.24	54.63
Central South Dakota	62.93	10.94	51.99
Western North Dakota	61.02	10.00	51.02
Central Minnesota	64.11	10.73	53.38
Northern Minnesota	63.23	10.82	52.41
Corn			
No. 1	71.12	5.99	65.13
No. 2	70.25	6.13	64.12
No. 3	69.52	6.61	62.91
No. 4	70.70	5.92	64.78
No. 5	71.11	6.31	64.80
No. 6	70.74	6.38	64.36

TABLE VI

CORN

Sample No.	Starch true fermentable substances	Alcohol yield			Difference between columns 4 and 5	Efficiency of fermentation
		Theoretical	Assuming 88% conversion	Actual		
	%	p.g./bu.	p.g./bu.	p.g./bu.	p.g./bu.	%
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	65.13	6.26	5.50	5.17	0.33	94.0
2	64.12	6.16	5.40	5.08	0.32	94.0
3	62.91	6.04	5.30	5.17	0.13	97.5
4	64.78	6.22	5.46	5.17	0.29	94.7
5	64.80	6.22	5.46	5.17	0.29	94.7
6	64.36	6.18	5.42	5.17	0.25	95.4

Each corn sample was fermented with the *Saccharomyces cerevisiae* strain of yeast and the yield of alcohol calculated. Table VI shows calculations of the theoretical proof gallons per bushel obtainable from the established true fermentable substances, the proof gallons per bushel expected with the assumption of 88% starch conversion as practically attainable, the difference in proof gallons per bushel between the yield from 88% starch conversion and the actual yield, and, finally, the efficiency of the fermentation. The starch content determined polarimetrically on these same samples in every case was higher than that of the true fermentable starch content, and fermentation efficiency calculated on this starch basis would be much lower than that shown in Table VI.

Discussion

By determining the pentosan content of grain and subtracting this figure from the starch content obtained by acid hydrolysis, a somewhat truer picture of the fermentable substances present in the grain was drawn than was otherwise possible by present methods. It must be held in mind that the term "pentosan" is used to include also other furfural-yielding materials. The hydrolysis products of these substances in addition to the converted starch reduced Fehling's solution and the total was expressed as dextrose, which was calculated back to starch. The pentosan content was then subtracted from this starch figure and the remainder was the fermentable figure.

The fermentable figure provides the chemist with a tool in assessing the value of grain. The utilization of this tool has been facilitated by the application of a rapid, modified bromine method for the determination of pentosans in grain and of an apparatus set-up especially designed for this purpose. The speed and accuracy of the method lend it to practical, industrial usage.

The modified excess-bromine method checked very well with the standard and itself as shown by duplicate runs on every sample. It is, therefore, worthy of consideration as an official method. It is more rapid than the standard method by approximately 38 hours and is accurate over a wider range of concentrations. A complete determination took about 100 minutes, which was even more rapid than that reported recently by Reeves and Munro (1940). The refluxing and extraction involved in their pentose determination consumed 150 minutes. A number of workers have reported that amounts of furfural less than 0.01 g. were not quantitatively determined by the precipitation method which either failed to indicate the presence of any furfural below this concentration or only a small fraction thereof, whereas the excess-bromine titration method was sensitive to such a small amount.

Sources of error in pentosan determinations such as loss of furfural by volatilization, decomposition of furfural by local superheating, and the use of rubber stoppers were avoided by the use of the special apparatus. The standard method of distillation allowed the distillate to drop from the end of the condenser into the receiver without special precaution against loss by volatilization. In the special apparatus the distillate was closed to air until the actual titration.

Corn and rye usually give approximately the same starch analysis by acid hydrolysis but the alcohol recovered by yeast fermentation from rye was much lower in comparison than that from corn. This fact was due to the presence of more substances unfermentable by yeast enzymes in rye than in corn as the analyses show in Table V. Control starch and pentosan analyses on grain are very important as shown by variations as much as 4.85% and 1.25%, respectively, on rye from eastern South Dakota and western North Dakota.

Rye from eastern North Dakota and eastern South Dakota was shown to contain the greatest amount of true fermentable substances.

The study of alcoholic fermentation made by Pasteur resulted in an alcoholic fermentation balance that was so authoritative that to date it has not been changed, although much controversy has arisen over it as presented in papers by Savary (1938, 1939) and Perard (1939). Pasteur found an alcoholic fermentation of cane sugar to yield alcohol, carbonic acid, glycerin, and succinic acid. It is not possible to convert starch completely to alcohol and an efficiency fermentation of 95%, based on the theoretical and actual yields, is optimum, according to this fermentation balance.

When we consider that all this work was empirical and that the study of truly quantitative analyses of the composition of grain is far from perfected, an effort toward the finding of satisfactory analytical methods would be well worth while. The yield of furfural, for example, was from a complex mixture and was derived from many sources. It was impossible to correlate the percentage of furfural obtained thus with the percentage of pure pentosan in the grain. In the starch acid hydrolysis method, the hydrolysate may have consisted of a mixture of hexoses, pentoses, uronic acids, and possibly other substances capable of reducing Fehling's solution. The reducing value obtained was the resultant of interacting and opposing effects and was expressed as dextrose. This value was translated to an actual term of starch, and by subtracting the value obtained for " pentosan " content we arrived at the empirical true content of starch. Preece (1931), in attempting to determine hemicelluloses, actually isolated and weighed them by a tedious and rather inaccurate method. Anderson and Krznarich (1935) and Krznarich (1940) have separated the hemicelluloses from oat hulls into

further components by repeated fractionation. These direct determinations are deserving of further study.

With our empirical methods, we do nonetheless obtain comparable data that are of great value.

Summary

The reaction of furfural in the distillate from 12% hydrochloric-acid distillation of grain with bromine at 0°C. over a period of 4 minutes was limited to the addition of one molecule of furfural to one molecule of bromine. Of several time intervals for the reaction to proceed, the 4-minute period proved optimum. The contents of original unfermentable substances in various grain samples were determined from the amount of furfural in the distillate, which was calculated from the determination of excess bromine in solution titrimetrically with sodium thiosulfate. This method checked very well with the standard phloroglucinol one for the determination of pentosans in grain. The application and advantages of a special apparatus for the determination of pentosans in grain are shown. A true representation of fermentable substances in grain has been established by subtracting this unfermentable content from the starch content obtained by the acid-hydrolysis method, which included both fermentable and unfermentable carbohydrates. This fermentable figure serves as an important tool for practical industrial control.

Acknowledgment

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THE COMPARATIVE BAKING QUALITIES OF STARCHES PREPARED FROM DIFFERENT WHEAT VARIETIES

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Alsberg (1935) mentioned the influence that starch might have upon flour quality and the need for investigational work directed toward a solution of this problem. As pointed out in a publication by Harris and Mason (1940) the starch in wheat flour has not received the consideration that it deserves from the standpoint of flour quality. This constituent is normally present in wheat flour in a concentration of at least 70% by weight and it is only reasonable to expect that some effect must be exerted upon baking strength by variations in starch properties due to wheat variety or environmental conditions during the growing season of the plant when the starch is being elaborated. To correlate starch differences with baking quality has been rather difficult, owing to the effect of other variables that are operative, such as wheat gluten, which is a very important factor in relation to baking strength. A number of papers have been published dealing with the results of viscosity, phosphorus, swelling, diastatic, and other determinations upon starches prepared from different wheats. Efforts have been made to correlate the results with flour quality. This work has been reviewed by Harris and Mason (1940) and will not be cited in the present instance. These studies, however, have been handicapped by the presence of other variables in the baking test that influence the quality of the loaf produced.

Sandstedt, Jolitz, and Blish (1939) published the results of a study conducted to ascertain the possibility of producing bread from synthetic doughs made from mixtures of gluten and starch with the usual baking ingredients. These researchers prepared gluten and starch from wheat flour and mixed them in suitable proportions to produce satisfactory bread. The doughs were mixed in the Hobart-Swanson with water, yeast, sugar, and salt in the usual proportions with the exception of sugar, which was present in 7% concentration. The doughs were fermented and baked, producing loaves of the customary size and appearance. The additional quantity of sugar was included in the formula to prevent yeast starvation during the fermentation period. This method, it would seem, could be employed, as pointed out by the authors, for the direct determination of starch quality effects upon baking strength since a uniform gluten substrate could

be used upon which different starches might be superimposed in a series of doughs. Differences in baking results obtained could then be attributed to variations in baking quality of the starches employed in making up the doughs.

Experimental Material and Methods

Starches were prepared from 20 flours which had been experimentally milled from varieties of the hard red spring, hard red winter, soft red winter, durum, and white wheat classes. These wheats were grown in the states of North Dakota, Kansas, Nebraska, Indiana, and Washington. The starch was separated from the gluten by washing under a small stream of 0.1% sodium phosphate solution. The washings containing the starch were strained through a 48-XX bolting cloth followed by a second straining through a 12-XX silk. In the latter instance, some time was required to work the starch suspension through the cloth. About 2,000 cc. of suspension was washed on the average from 400 g. of flour. The quantity of flour used varied from 248 g. to 400 g., depending upon the amount available for the investigation.

After the double straining, the starch was allowed to settle out in the cold and the supernatant liquid decanted from the residual starch. The starch was centrifuged, and the separated liquid again decanted from the moist starch and a supernatant gelatinous layer. This layer was tentatively named "amylodextrin" by Sandstedt, Jolitz, and Blish (1939)—inasmuch as it appeared that this material had been formed from soluble starch by the action of beta-amylase. The same term will be used to denote this substance in the present paper. The starch and amylodextrin were removed from the centrifuge tubes and dried in trays under a fan at 90° to 94°F. with occasional turning and pressing with a spatula, to facilitate the removal of moisture and to break up the lumps which formed during drying. The dried starch was finally run through a small wheat grinder to reduce any lumps which had formed while the starch was drying. The sample was thoroughly mixed and the moisture and nitrogen content determined.

A series of preliminary experiments was made to determine the best method to use for the purpose of obtaining optimum baking results. It was decided to omit entirely the sponge procedure of preparation and to investigate the "soaker" method. Both of these methods were developed by Sandstedt, Jolitz, and Blish (1939). The procedures used and the baking results yielded by these variations of gluten treatment and mixing times are shown in Table I. The first attempt made to incorporate dry gluten and starch into a dough without an initial period of gluten hydration resulted in an inferior

TABLE I

METHOD OF GLUTEN HYDRATION AND MIXING TIMES EMPLOYED
IN THE PRELIMINARY STUDY INCLUDING BAKING DATA

Protein level of starch-gluten mixture 13.2% (13.5% moisture basis).

No. of procedure	Method employed Gluten hydrated at 30°C.	Mixing time	Loaf volume	Texture[1]	Crumb color[2]	Crust[3]	Symmetry[4]
		min.	*cc.*				
I	Gluten and starch mixed dry.	2½	150	5.0 c	5.0 g-y	S	2.5
II	Gluten worked into a ball with a quantity of water and allowed to soak 1 hour; then excess water squeezed out, ball broken into small pieces and mixed with the starch.	1 2 2½	155 165 200	5.5 C, o 6.5 o 7.0	5.0 g-y 6.0 g-y 7.0	Du S S	3.5 4.0 4.5
III	12 cc. of water added to the gluten in a 30-cc. beaker and allowed to soak (without agitation) for 1 hour; formed into ball; pressed out flat and cut into long thin strips and mixed with the starch.	1 2 2½	165 185 205	6.0 C, o 6.5 o 7.5	5.0 g-y 6.0 g-y 7.0	Du S S	3.5 4.0 4.5

[1] Texture: o = open, C = coarse, c = close. Perfect score = 10.
[2] Color: g-y = gray-yellow. Perfect score = 10.
[3] Crust: Du = dull, S = satisfactory.
[4] Symmetry: Perfect score = 5.

loaf. The next method tried involved forming a gluten ball which stood for one hour at 30°C. The ball was then broken into small pieces and mixed with the starch. Mixing periods of 1 to 2½ minutes were used, the best results being obtained with the 2½-minute mix. The next trial was made by adding a definite quantity (12 cc.) of distilled water to the gluten and soaking for one hour. The hydrated gluten was then formed into a ball with the fingers, pressed flat and cut into strips which were incorporated with the dry starch into a dough. In this instance, 2½ minutes of mixing again gave optimum results. As this method of gluten hydration with 2½-minute mixing gave the best results, it was adopted for use in the starch-quality investigations. The use of periods shorter than one hour for gluten hydration resulted in a "granular" feeling dough, in which the original gluten particles could be observed, indicating apparently imperfect gluten hydration.

The Hobart mixer, equipped with two dough hooks, was used for mixing the synthetic doughs, employing the medium speed for 30 seconds. The mixer was then stopped and the gluten and starch scraped down from the sides of the bowl and the mixing pins. Fifteen seconds was allowed for this operation. The mixer was again started and low-speed mixing conducted until the stop watch registered a total of 2½ minutes from the time when the mixer was first started. Thus

a total mixing time of 2½ minutes, less 15 seconds, was used. The gluten and starch were found to mix quite readily and no indications were noticed of incomplete incorporation of the gluten in the dough. The loaves were quite normal in appearance, color, and texture. The micro baking method described by Geddes and Aitken (1935) and used by Harris and Sanderson (1939), as well as Van Scoyk (1937, 1939), was employed in this investigation, and proved to be quite satisfactory from the standpoint of results obtained. This method was especially suitable because of the relatively small quantities of starch and gluten required to produce a dough as compared with the older methods which necessitate the use of much larger doughs. The formula used was the malt-phosphate-bromate with 7% of sucrose included to insure the presence of sufficient fermentable material during the dough-fermentation period. A standard period of 3 hours of fermentation previous to panning with 55 minutes in the pan was allowed. The usual methods of handling the dough were followed.

The dried, powdered gluten used in this study was prepared from a commercial hard red spring wheat flour (13.2% protein) by the method described by Harris (1940). This gluten contained 10.1% moisture and 69.8% crude protein (N × 5.7) on a 13.5% moisture basis. Gluten in this form is more convenient to use than wet crude gluten, as pointed out by Aitken and Geddes (1938) and also by Harris (1940), although some evidence of alteration in the biochemical properties of the gluten occasioned by drying have been found by the latter.

Three protein levels, 10.0%, 13.2%, and 16.0% (13.5% moisture basis), were selected for the starch-gluten mixtures. It is probable that the gluten protein content of these levels would be somewhat above that of flours of equivalent total protein content because of the inclusion of other than gluten protein in the latter value. The proportions of prepared dried starch and gluten necessary to produce a synthetic flour at these three protein contents were calculated, taking into account the respective protein contents of the dried gluten and starch. The quantity of gluten required for each dough was weighed out and placed in a small beaker under distilled water for one hour. For the lower protein levels of 10.0% and 13.2%, 12 cc. of distilled water was used. In the instance of the 16.0% protein level, 15 cc. of distilled water was used. All water added was at a temperature of 30°C. and allowed to stand for one hour to hydrate the gluten. The necessary quantity of starch was weighed out and placed in a suitably numbered container. The container and contents were then placed in a cabinet at 30°C. until required for mixing with the gluten. The entire quantity of water added to the glutens was later transferred to the mixing bowl.

Discussion of Results

A description of the wheats from which the starches were prepared with comparative milling and analytical data are presented in Table II. The wheat protein covered a range of 7.6%, while the flour

TABLE II

DESCRIPTIONS AND COMPARATIVE DATA OBTAINED ON THE WHEATS FROM WHICH STARCHES USED IN THE BAKING INVESTIGATIONS WERE PREPARED

Results arranged in order of increasing wheat protein content. All samples of wheat grown in 1939 except Nos. 1, 5, 7, and 20, which were grown in 1938.

Sample No.	Class and variety[1]	State grown	Test weight per bushel	Crude protein (N ×5.7) Wheat	Crude protein (N ×5.7) Flour	Total flour yield	Flour ash
			lbs.	%	%	%	%
1	SRW, Trumbull	Ind.	63.8	10.0	8.5	72.3	0.48
2	SRW, Wabash	Ind.	64.2	10.1	8.6	66.6	0.48
3	SW, Federation	Wash.	60.8	10.8	9.3	66.0	0.40
4	HW, Early Baart	Wash.	63.5	11.1	9.7	65.9	0.44
5	HRW, Chiefkan	Kan.	62.7	11.2	10.5	76.3	0.48
6	HRS, Vesta	N. D.	61.2	11.6	11.3	73.5	0.48
7	HRW, Kanred	Kan.	58.0	12.8	12.3	74.0	0.51
8	HRW, Tenmarq	Kan.	61.5	14.1	13.2	63.3	0.38
9	HRS, Mercury	N. D.	56.3	14.2	13.4	70.2	0.56
10	HRS, Rival	N. D.	55.5	15.2	14.0	70.6	0.43
11	E, Vernal	N. D.	31.8	15.2	16.8	47.2	0.68
12	D, Mindum	N. D.	60.2	15.3	14.6	69.9	0.56
13	HRS, Premier	N. D.	58.2	15.3	15.0	70.3	0.54
14	HRW, Chiefkan	Kan.	61.8	15.8	15.3	73.0	0.43
15	HRW, Cheyenne	Nebr.	63.2	16.2	14.6	63.5	0.47
16	HRW, Blackhull	Kan.	61.0	16.2	15.8	68.5	0.45
17	HRW, Nebred	Nebr.	59.8	16.9	16.2	68.3	0.42
18	HRS, Thatcher	N. D.	56.6	16.9	16.6	69.0	0.51
19	HRW, Turkey	Kan.	61.5	17.2	16.9	69.5	0.58
20	D, Mindum	N. D.	60.8	17.6	17.1	75.6	0.88

[1] SRW = soft red winter. SW = soft white. HRW = hard red winter. HRS = hard red spring. E = emmer. D = durum.

protein covered a range of 8.6%. There was also a marked variability in total flour yield, as would be expected with milling wheats as variable in nature as those encountered in this investigation. It will be noticed that the Tenmarq from Kansas produced the lowest-ash flour, while the new Nebred, grown in Nebraska, produced the second-lowest. Several of the wheats produced relatively high-ash flours, such as Vernal emmer, the two samples of durum grown in North Dakota, and Turkey from Kansas.

In Table III are shown the comparative baking data obtained on the flours. The absorption shows substantial differences between the various samples, being lowest for Vernal emmer and highest for Mindum. The soft winter wheats, as a whole, ran lower than the hard wheats in water-absorbing capacity. The loaf volumes varied from

TABLE III

COMPARATIVE BAKING DATA OBTAINED ON THE FLOURS
FROM WHICH THE STARCHES WERE PREPARED

Results arranged in order of increasing wheat protein content.

Sample No.	Class and variety[1]	Absorption	Loaf volume	Texture[2]	Crumb color[3]	Crust[1]	Symmetry[5]
		%	cc.				
1	SRW, Trumbull	58.0	469	7.4	7.2	P	2.5 o
2	SRW, Wabash	51.3	485	6.5 o	7.0 y	P	2.5 o
3	SW, Federation	52.2	440	3.5 C, o	5.0 g-y	P	2.0 o
4	HW, Early Baart	55.6	510	6.5 o	7.0 y	P	2.5 o
5	HRW, Chiefkan	58.0	510	3.0 C, o	5.0 g-y	Du	2.5 o
6	HRS, Vesta	57.8	490	6.0 C, o	6.8 y	S	3.0 o
7	HRW, Kanred	58.0	616	7.2	7.3	S	3.5 o
8	HRW, Tenmarq	54.0	595	7.5	8.2	S	4.0 o
9	HRS, Mercury	58.1	680	7.1	7.3	SlD	4.4 o
10	HRS, Rival	59.6	685	7.5	7.8	D	4.3 o
11	E, Vernal	49.8	510	3.0 C, o	3.5 g	Du	2.0
12	D, Mindum	54.6	490	4.5 C, o	4.5 y	Du	2.5 o
13	HRS, Premier	58.1	685	7.5	7.5	SlD	4.5 o
14	HRW, Chiefkan	58.4	655	6.5 o	7.5	S	4.2 o
15	HRW, Cheyenne	55.1	655	7.2	7.8	S	4.0 o
16	HRW, Blackhull	55.7	715	7.0 o	7.8	S	4.5 o
17	HRW, Nebred	53.8	780	7.0 o	7.2	S	4.5 o
18	HRS, Thatcher	59.6	910	6.2 o	7.0 y	SlD	4.5 o
19	HRW, Turkey	55.3	720	6.5 o	7.0 y	S	4.5 o
20	D, Mindum	63.0	465	7.3	7.0	Du	2.5 o

[1] See footnote, Table II.
[2] Texture: o = open, C = coarse, c = close. Perfect score = 10.
[3] Color: y = yellow, g-y = gray-yellow, g = gray. Perfect score = 10.
[4] Crust: Du = dull, S = satisfactory, SlD = slightly dark, P = pale.
[5] Symmetry: o = overoxidized. Perfect score = 5.

440 to 910 cc., a range of 470 cc., while other characteristics of the loaves likewise showed large variations between the various flours. A series of flours milled from wheats embracing such disparity in baking strength and grown under marked differences of environmental conditions, should form excellent material for the preparation of starches to be used in a study of the effects of starch differences upon baking strength.   If wheat starches differ in baking characteristics, these differences should be apparent in a study conducted with variable material of this nature.

It may be pointed out that several of the hard red spring wheats included in this investigation are quite new.   Mercury is one of these and has been found to be below the average of spring wheat in baking quality and has, accordingly, not been distributed to the grain growers of North Dakota.   Vesta is another new variety that has been under consideration for distribution, but has not yet been recommended. Premier is a third recently developed variety, and no decision has been made in respect to its release.   Rival is considered to be higher in baking strength than the other spring wheats listed, with the exception

of Thatcher, which is a very strong wheat. From results obtained in this laboratory when the 1939 crop was examined, these wheats would rank as follows in order of decreasing baking strength: Thatcher, Rival, Vesta, Premier, and Mercury.

In Table IV are shown the moisture and protein contents of the starches prepared from this series of wheats. A substantial variation

TABLE IV

CLASS AND VARIETY OF WHEAT FROM WHICH STARCH WAS PREPARED AND MOISTURE AND PROTEIN CONTENT OF STARCH

Sample No.	Class and variety	Starch	
		Moisture	Protein[1]
		%	%
1	Soft red winter, Trumbull	10.8	0.44
2	Soft red winter, Wabash	10.8	0.49
3	Soft white, Federation	10.9	0.34
4	Hard white, Early Baart	11.1	0.40
5	Hard red winter, Chiefkan	11.9	0.48
6	Hard red spring, Vesta	12.1	0.47
7	Hard red winter, Kanred	10.8	0.52
8	Hard red winter, Tenmarq	10.6	0.45
9	Hard red spring, Mercury	11.5	0.71
10	Hard red spring, Rival	11.8	0.59
11	Emmer, Vernal	9.0	0.45
12	Durum, Mindum	9.0	0.56
13	Hard red spring, Premier	12.5	0.53
14	Hard red winter, Chiefkan	8.9	0.44
15	Hard red winter, Cheyenne	10.6	0.52
16	Hard red winter, Blackhull	9.6	0.41
17	Hard red winter, Nebred	9.1	0.51
18	Hard red spring, Thatcher	13.6	0.65
19	Hard red winter, Turkey	8.9	0.51
20	Durum, Mindum	10.9	0.83

[1] Protein calculated (N × 5.7) on 13.5% moisture basis.

in moisture content is noticeable among the various starch samples, ranging from 9.0% to 12.5%—a difference of 3.5%, but whether this is due to the inherent characteristics of the starch is somewhat doubtful as no particular care was taken during drying to insure a constant moisture level in the samples. There does seem to be some indication, however, of a higher moisture content in the starches prepared from the hard red spring wheats. The protein content of the starches was as constant as one would expect, considering the method of preparation. No attempt was made to extract any residual protein from the starches by the use of protein solvents.

Table V presents the comparative baking data obtained from the synthetic starch-gluten doughs mixed and baked as described. These data are on a 13.5% moisture basis and the samples have been arranged in order of decreasing loaf volume within classes to correspond with the

photographs of the loaves. The average loaf volumes and loaf scores are presented in Table VI. The absorption tended to increase with the protein level of the dough as would be expected. The 16.0%-protein-level doughs were very high in absorption, ranging up to 77.7% for the blend made with Mindum wheat starch, sample No. 20. The two durum wheat starches produced doughs with the highest absorptions. It is also quite clear that loaf colume increased with protein content in every instance, showing that in synthetic doughs made from dried starch and gluten, protein content is an important

TABLE V

BAKING DATA OBTAINED FROM SYNTHETIC STARCH-GLUTEN
DOUGHS AT THREE PROTEIN LEVELS

Data arranged in order of decreasing loaf volume within classes.

Sample No.	Variety	Absorption— protein levels of			Loaf volumes— protein levels of			Texture[1]—protein levels of		
		10.0%	13.2%	16.0%	10.0%	13.2%	16.0%	10.0%	13.2%	16.0%
					cc.	cc.	cc.			
		HARD RED SPRING VARIETIES								
18	Thatcher	60.7	61.7	63.7	165	185	215	5.0 C, o	5.0 C, o	5.5 o
10	Rival	64.7	64.7	67.7	145	165	210	7.0	7.0	7.0
9	Mercury	63.7	63.7	66.7	145	170	205	4.5 c	6.0	7.0
13	Premier	63.7	63.7	66.7	150	165	195	6.0	6.0	7.0
6	Vesta	63.7	63.7	66.7	105	155	170	3.5 c	4.5 c	6.0
		HARD RED WINTER VARIETIES								
17	Nebred	65.7	67.7	69.7	142	175	205	5.5 o	6.5 o	7.0
15	Cheyenne	67.7	69.7	71.7	140	155	200	5.5 o	6.5 o	7.0
16	Blackhull	66.7	67.7	69.7	135	165	180	4.5 c	6.5 o	6.5 o
19	Turkey	67.7	69.7	71.7	125	155	180	4.5 c	5.5 c	6.0
14	Chiefkan	67.7	69.7	71.7	135	155	175	4.5 o	6.5 o	7.0
8	Tenmarq	63.7	69.7	71.7	120	155	165	4.5 c	5.5 c	6.5 o
		MISCELLANEOUS VARIETIES								
2	Wabash	62.7	64.7	68.7	178	210	255	6.5 o	7.0	7.0
11	Vernal emmer	69.7	69.7	71.7	150	172	215	5.5 o	6.5 o	7.0
12	Mindum	68.7	72.7	73.7	132	165	200	4.5 c	6.5 o	7.0
3	Federation	61.7	63.7	67.7	135	155	185	4.5 c	6.5 o	7.0
4	Early Baart	63.7	63.7	67.7	125	152	175	4.5 c	5.5 c	6.0 o
		VARIETIES GROWN IN 1938								
7	Kanred	66.7	67.7	69.7	158	185	215	5.5 o	6.5 o	7.0
5	Chiefkan	71.7	71.7	71.7	150	175	215	5.5 o	7.0 o	6.5 o
1	Trumbull	65.7	67.7	69.7	140	195	200	5.5 o	7.0	7.0
20	Mindum	75.7	77.7	77.7	130	150	198	4.5 c	6.5 o	7.0

TABLE V—*Continued*

Sample No.	Variety	Crumb color[2]— protein levels of			Crust color[3]— protein levels of			Symmetry[4]— protein levels of		
		10.0%	13.2%	16.0%	10.0%	13.2%	16.0%	10.0%	13.2%	16.0%
		HARD RED SPRING VARIETIES								
18	Thatcher	4.5 g-y	5.5 g-y	6.0 g-y	Du	S	S	2.0 o	3.0 o	4.5 o
10	Rival	6.0 g-y	6.5 g-y	6.5 g-y	P	S	S	2.0 o	4.0 o	4.5 o
9	Mercury	5.5 g-y	5.5 g-y	6.5 g-y	P	Du	S	2.0 o	3.0 o	4.5 o
13	Premier	5.0 g-y	6.0 g-y	6.5 g-y	P	Du	S	2.0 o	3.0 o	4.5 o
6	Vesta	4.0 g-y	5.5 g-y	5.5 g-y	P	Du	Du	1.0 o	2.0 o	4.0 o
		HARD RED WINTER VARIETIES								
17	Nebred	6.0 g-y	6.5 g-y	6.5 g-y	P	Du	S	2.0 o	3.5 o	4.5 o
15	Cheyenne	5.5 g-y	6.5 g-y	6.5 g-y	P	S	S	2.0 o	3.0 o	4.5 o
16	Blackhull	5.5 g-y	6.5 g-y	6.5 g-y	P	S	S	1.0 o	3.5 o	3.5 o
19	Turkey	6.0 g-y	6.0 g-y	6.5 g-y	P	P & Du	Du	1.0 o	2.5 o	3.5 o
14	Chiefkan	6.0 g-y	6.5 g-y	6.5 g-y	P	Du	S	2.0 o	2.5 o	3.5 o
8	Tenmarq	5.0 g-y	6.0 g-y	6.0 g-y	P	P & Du	Du	1.0 o	2.5 o	3.5 o
		MISCELLANEOUS VARIETIES								
2	Wabash	6.5 g-y	6.5 g-y	7.0 y	Du	S	S	4.0 o	4.0 o	4.5 o
11	Vernal emmer	6.0 g-y	6.5 g-y	6.5 g-y	S	Du	S	3.5 o	3.5 o	4.5 o
12	Mindum	6.0 g-y	6.5 g-y	6.5 g-y	Du	Du	S	2.0 o	3.5 o	4.0 o
3	Federation	6.5 g-y	6.5 g-y	6.0 g-y	P	P & Du	Du	2.0 o	3.0 o	4.0 o
4	Early Baart	5.0 g-y	6.0 g-y	6.0 g-y	P	P & Du	Du	1.0 o	3.0 o	4.0 o
		VARIETIES GROWN IN 1938								
7	Kanred	6.5 g-y	7.0 y	7.0 y	Du	S	S	3.5 o	3.5 o	4.5 o
5	Chiefkan	5.5 g-y	7.0 y	6.5 g-y	S	S	S	3.0 o	4.0 o	4.5 o
1	Trumbull	6.5 g-y	6.5 g-y	6.5 g-y	P	Du	S	2.0 o	4.0 o	4.5 o
20	Mindum	5.5 g-y	6.5 g-y	6.5 g-y	Du	S	S	2.0 o	3.0 o	3.5 o

[1] Texture: o = open, C = coarse, c = close. Perfect score = 10.
[2] Color: y = yellow, g-y = gray-yellow, g = gray. Perfect score = 10.
[3] Crust: Du = dull, S = satisfactory, SlD = slightly dark, P = pale.
[4] Symmetry: o = overoxidized. Perfect score = 5.

factor in determining the loaf volume. The protein added was, of course, the same sample throughout and therefore differences due to variations in protein *quality* are eliminated. The texture and symmetry scores were also increased by increasing the gluten content.

The highest loaf volume at all protein levels was produced by starch from a soft red winter wheat, Wabash. These loaves were of excellent crumb color, texture, and symmetry. The doughs mixed from the starch from this variety were firm and elastic and showed every indication during handling of producing superior bread. The starch next in order from the standpoint of baking performance was prepared from wheat No. 18, Thatcher, a hard red spring variety,

TABLE VI

AVERAGE ABSORPTION AND LOAF VOLUME WITH CRUMB COLOR, TEXTURE, AND SYMMETRY SCORES OF STARCH-GLUTEN BLENDS

Data arranged in order of decreasing loaf volume within classes.

Sample No.	Variety	Absorp-tion	Loaf volume	Texture[1]	Crumb color[1]	Crust color[1]	Sym-metry[1]
		%	cc.				
		HARD RED SPRING VARIETIES					
18	Thatcher	62.0	188	5.2 C, o	5.3 g-y	S	3.2 o
10	Rival	65.7	173	7.0	6.3 g-y	S	3.5 o
9	Mercury	64.7	173	5.8 o	5.8 g-y	Du	3.2 o
13	Premier	64.7	170	6.3	5.8 g-y	Du	3.2 o
6	Vesta	65.0	143	4.7 c	5.0 g-y	Du	2.3 o
		HARD RED WINTER VARIETIES					
17	Nebred	67.7	174	6.3 o	6.3 g-y	Du	3.3 o
15	Cheyenne	69.7	165	6.3 o	6.2 g-y	S	3.2 o
16	Blackhull	68.0	160	5.8 o	6.2 g-y	S	2.7 o
19	Turkey	69.7	153	5.3 c	6.2 g-y	Du	2.3 o
14	Chiefkan	69.7	155	6.0 o	6.3 g-y	Du	2.7 o
8	Tenmarq	68.4	147	5.5 c	5.7 g-y	Du	2.3 o
		MISCELLANEOUS VARIETIES					
2	Wabash	65.4	214	6.8 o	6.7 g-y	S	4.3 o
11	Vernal emmer	70.4	179	6.3 o	6.3 g-y	Du	3.8 o
12	Mindum	71.7	166	6.0 o	6.3 g-y	Du	3.2 o
3	Federation	64.4	158	6.0 o	6.3 g-y	Du	3.0 o
4	Early Baart	65.0	151	5.3 c	5.7 g-y	Du	2.7 o
		WHEATS GROWN IN 1938					
7	Kanred	68.0	186	6.3 o	6.8 y	S	3.8 o
5	Chiefkan	71.7	180	6.3 o	6.3 g-y	S	3.8 o
1	Trumbull	67.7	178	6.5 o	6.5 g-y	Du	3.5 o
20	Mindum	77.0	159	6.0 o	6.2 g-y	S	2.8 o

[1] For explanation of letters used in baking scores, refer to footnote on Table V.

with Kanred starch next in quality ranking. Vernal emmer starch produced an excellent loaf at the higher protein concentrations. The dough made from the Thatcher starch blends comported itself quite differently from the doughs prepared from other starch samples in the baking test, forming a sticky, unsatisfactory dough which gradually became firm and more consistent as the fermentation progressed. In this manner the dough was similar to doughs made from certain samples of spring wheat flour that are slack and loose after mixing but which tighten up and produce satisfactory loaves of bread. Another feature of this investigation was the excellent loaves produced by starch

prepared from the hard red winter variety, Chiefkan, which was grown in 1938. The other Chiefkan sample, No. 14, grown in 1939, produced a starch distinctly lower in baking quality as judged by these tests. Vesta wheat starch was comparatively poor in performance, with Tenmarq, Early Baart, and Turkey increasing in value in the order named. Mindum wheat produced in 1938 and 1939 did not show great differences in starch quality in these tests.

Photographs of the loaves produced from the different starch-gluten blends are shown in Figures 1 to 8. These photographs, which are arranged in order of decreasing loaf volume by classes, emphasize the points already brought out in this discussion.

Figures 1 and 2 show the exterior and interior appearance of the loaves baked from starches prepared from hard red spring wheat varieties and dried gluten. In the higher protein range the loaves were of a strong, bold appearance with an even break and shred. In the instance of the lower protein levels the loaves had an overoxidized appearance, but this characteristic disappeared when the protein content was increased to 16.0%. Thatcher wheat starch produced loaves with a tendency toward an open texture, but the loaf was quite elastic with thin cell walls; the other hard red spring wheat starches yielded loaves that were better than Thatcher in texture. Vesta starch baked into the poorest loaf shown in Figures 1 and 2, especially at the 10.0% protein level. The crumb color was very good for all the loaves with the exception of Vesta, which was inferior.

The loaves baked from the hard red winter starch blends are shown in Figures 3 and 4. These starch-gluten blends all produced bold, symmetrical loaves at the 16.0% protein level. Loaves made from Nebred and Cheyenne were the strongest in appearance. The crust was very satisfactory except in the instance of Turkey and Tenmarq, which had a dull sheen. The textures were all good in the high protein blends, and the crumb colors could be classified as fair.

The loaves obtained from blends made with starches from a miscellaneous group of varieties are shown in Figures 5 and 6. As pointed out in the instance of the spring-wheat varieties, the loaves from the high-protein blends lack the overoxidized appearance of the lower-protein blends. The loaves in the high-protein level were bold, with an even break and shred. The crust color was satisfactory except for the loaves prepared from the white-wheat starches, which were decidedly dull. Textures were good in this group. The crumb colors were on a lower level than in the preceding groups. Remarkably large, bold loaves were produced from the Wabash-starch blends.

Figures 7 and 8 show loaves baked from hard and soft red winter and white-wheat starch blends. It is interesting to note that the two

blends of soft-wheat starch produced larger loaves than the two blends of hard-wheat starch at all protein levels.   In the case of the red winters the soft-wheat blends exceeded the hard-wheat-starch doughs in loaf volume by 58 cc. at the 10% level, by 55 cc. at the 13.2% level,

PROTEIN LEVELS

10.0%   13.2%   16.0%

STARCH
from

Thatcher

Rival

Mercury

Premier

Vesta
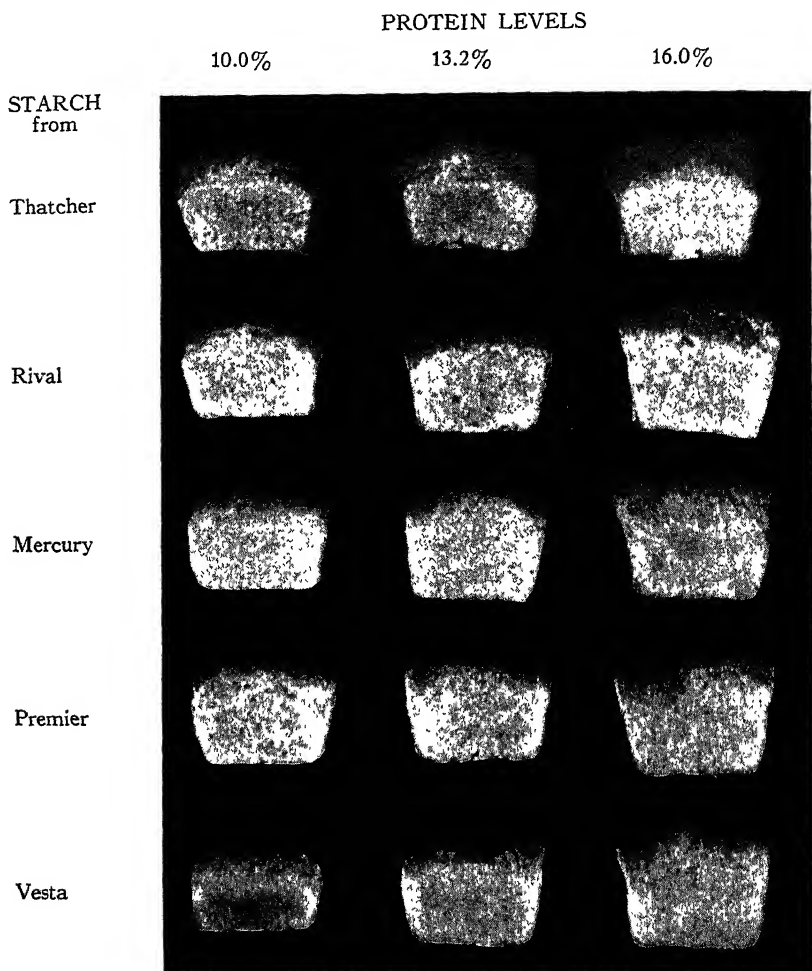
Fig. 1.   Outward appearance of loaves baked from blends of hard red spring wheat starch with a common gluten substrate.

and by 75 cc. at the 16.0% level.   The loaves produced with starch from the soft red winter wheat were bold, with even symmetry and good crust color.   The hard red winter blends, however, yielded loaves of poor volume and ragged appearance with a tendency toward flat

top at the lower protein levels.   The 16.0% protein loaf was considerably better, but only equal to the loaf from the 10.0% protein soft red winter blend.   The crust colors were also pale and dull in the loaves made with hard wheat starch.   The texture and crust color increased

PROTEIN LEVELS

| | 10.0% | 13.2% | 16.0% |

STARCH from

Thatcher

Rival

Mercury

Premier

Vesta
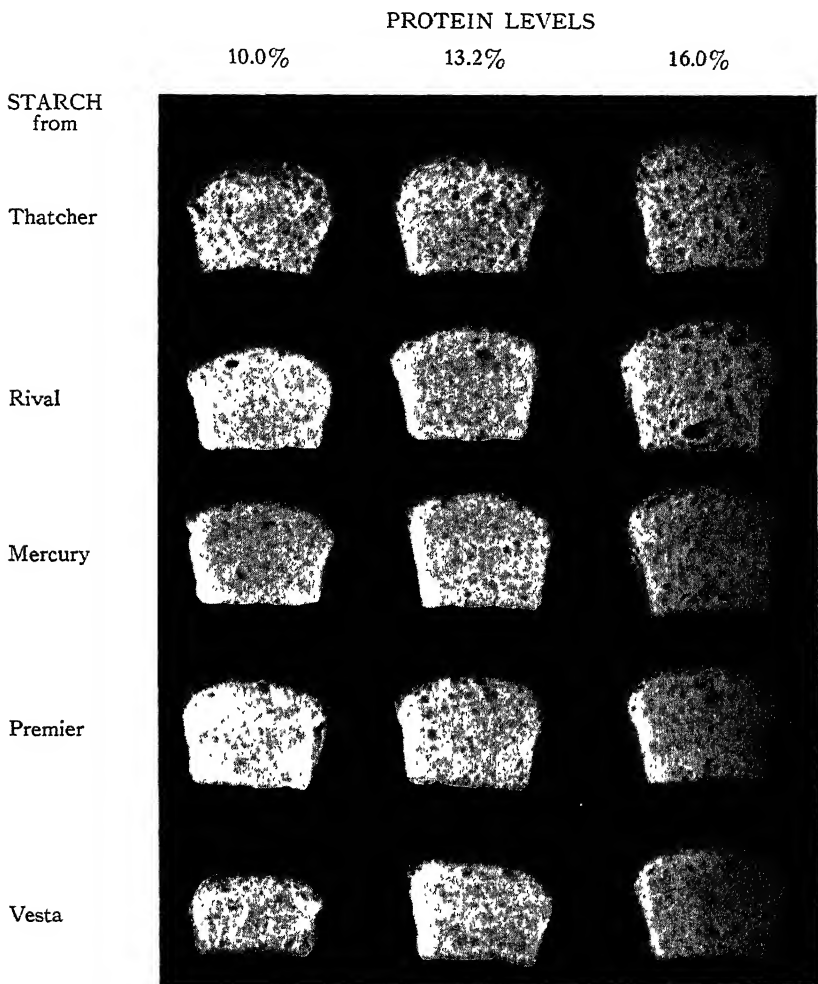
Fig. 2.   Cut surfaces of loaves baked from blends of hard red spring wheat starch with a common gluten substrate.

with protein content in both wheat classes, but both of these loaf characters were decidedly better in the blends of soft red winter wheat starch.

In the comparison between the hard and soft white wheat, the
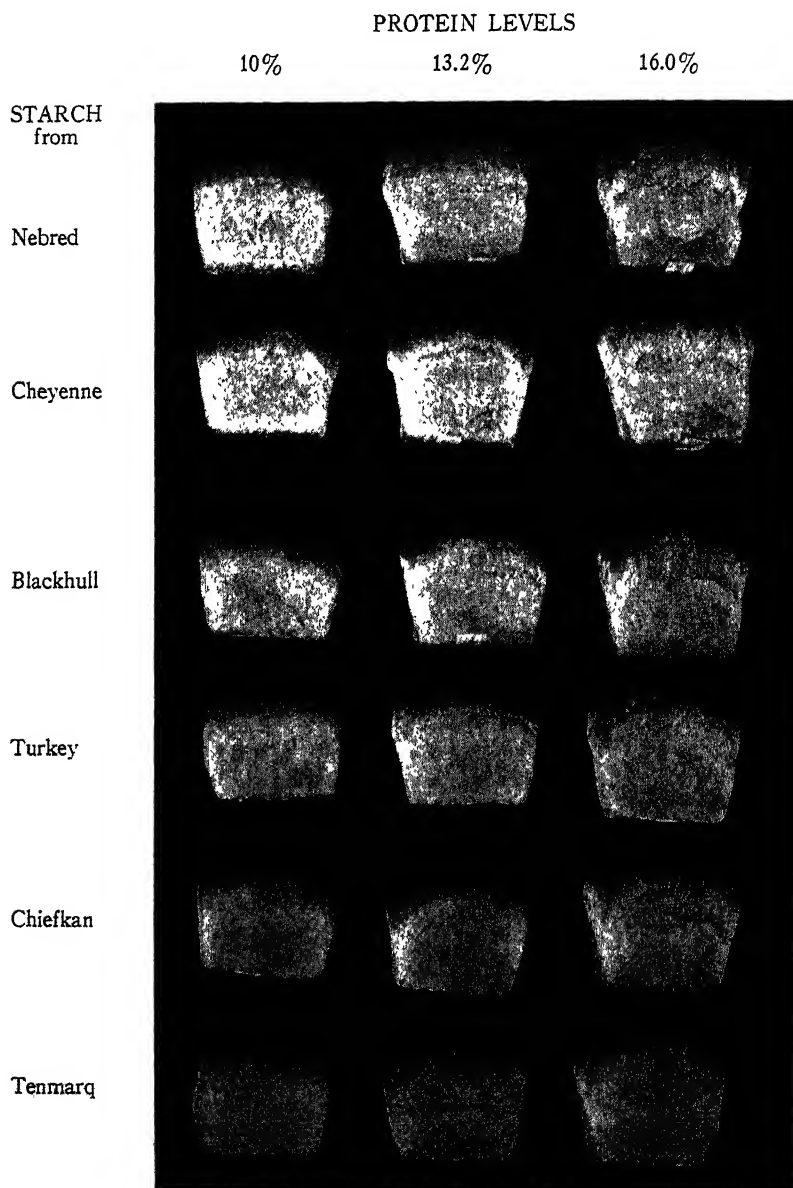
Fig. 3.   Outward appearance of loaves baked from blends of hard
red winter wheat starch with a common gluten substrate.
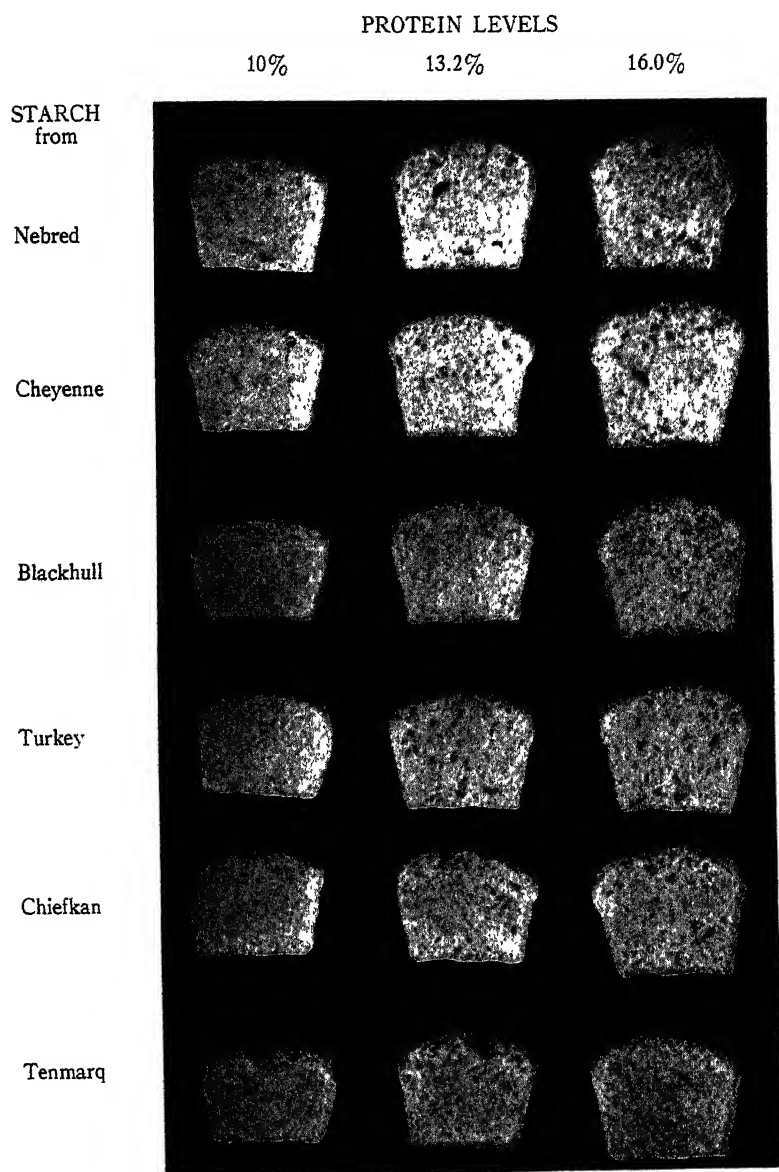
PROTEIN LEVELS

Fig. 4. Cut surfaces of loaves baked from blends of hard red winter wheat starch with a common gluten substrate.

soft-wheat starch again yielded better loaves than the hard-wheat starch at equivalent protein levels, but the difference between the two series of loaves was not so great as between the hard and soft red winter wheat starches.

PROTEIN LEVELS

| | 10.0% | 13.2% | 16.0% |

STARCH
from

Wabash

Vernal
emmer

Mindum

Federation

Early Baart
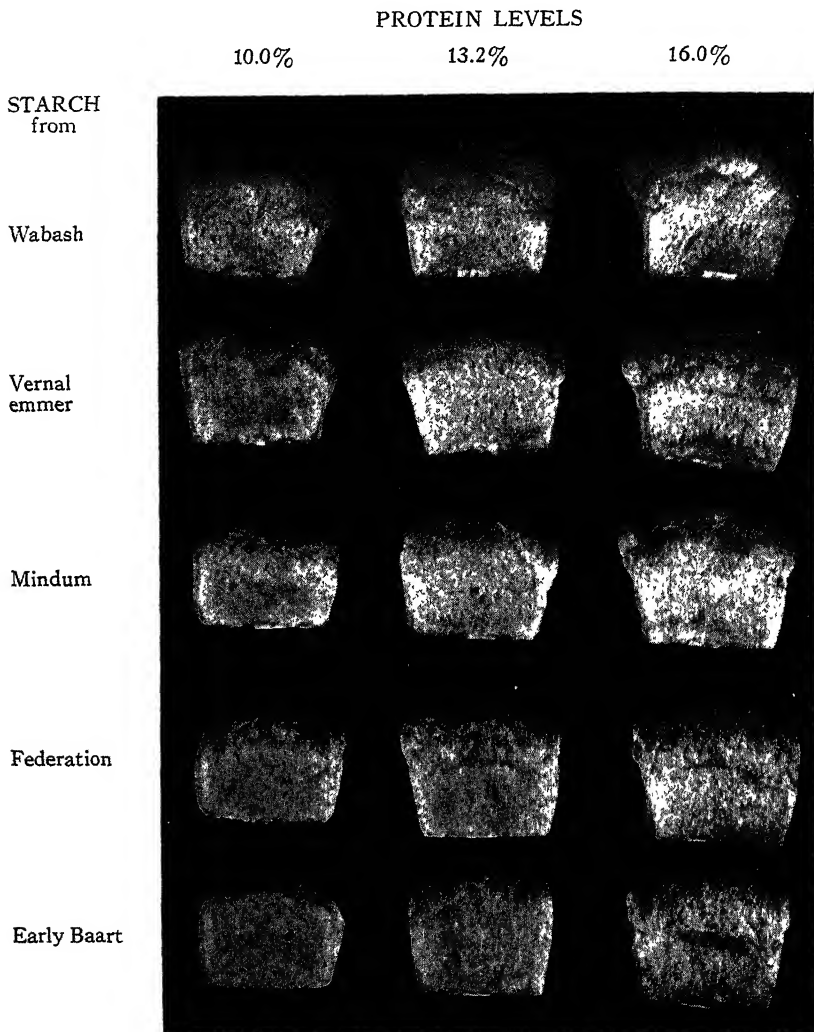
Fig. 5.   Outward appearance of loaves baked from blends of different wheat starches with a common gluten substrate.

It would appear that these characteristic differences in baking quality between hard and soft starch blends might be attributed, in part at least, to a greater degree of starch damage during milling in the

case of the hard wheats, as pointed out by Sandstedt, Jolitz, and Blish (1939).

PROTEIN LEVELS

	10.0%	13.2%	16.0%

STARCH
from

Wabash

Vernal
emmer

Mindum

Federation

Early Baart
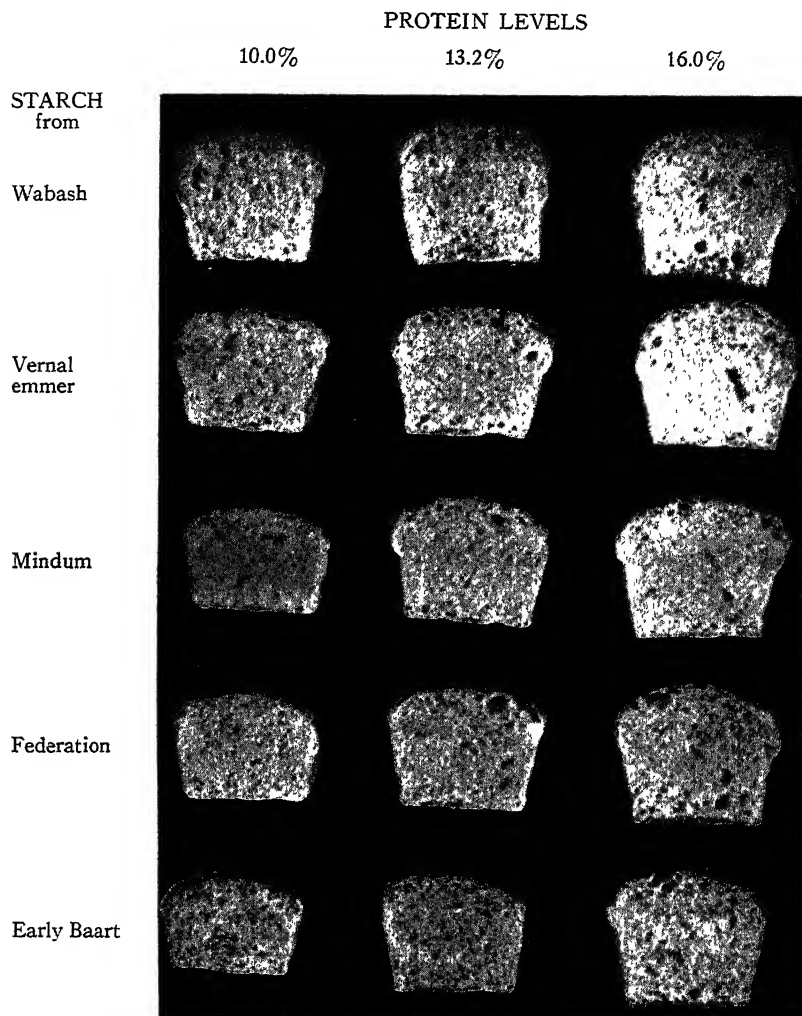
Fig. 6.   Cut surfaces of loaves baked from blends of different wheat
starches with a common gluten substrate.

Summary and Conclusions

The method of baking dry starch-gluten mixtures developed by Sandstedt, Jolitz, and Blish (1939) was applied to the determination of the baking quality of the starches prepared from a series of wheats embracing samples of the hard red spring, hard red winter, soft red

winter, durum, and white wheat classes.   A constant gluten substrate
prepared from hard red spring wheat was used in all the bakings.   An
initial period of one hour was allowed for the gluten to become hydrated
before mixing with the starch.   A mixing period of 2½ minutes was
used and gave satisfactory results.   Three protein levels were used
for the mixes: 10.0%, 13.2%, and 16.0% on a 13.5% moisture basis.
With these various protein levels, information regarding the differential
reaction of the starch-gluten blends to different protein contents could
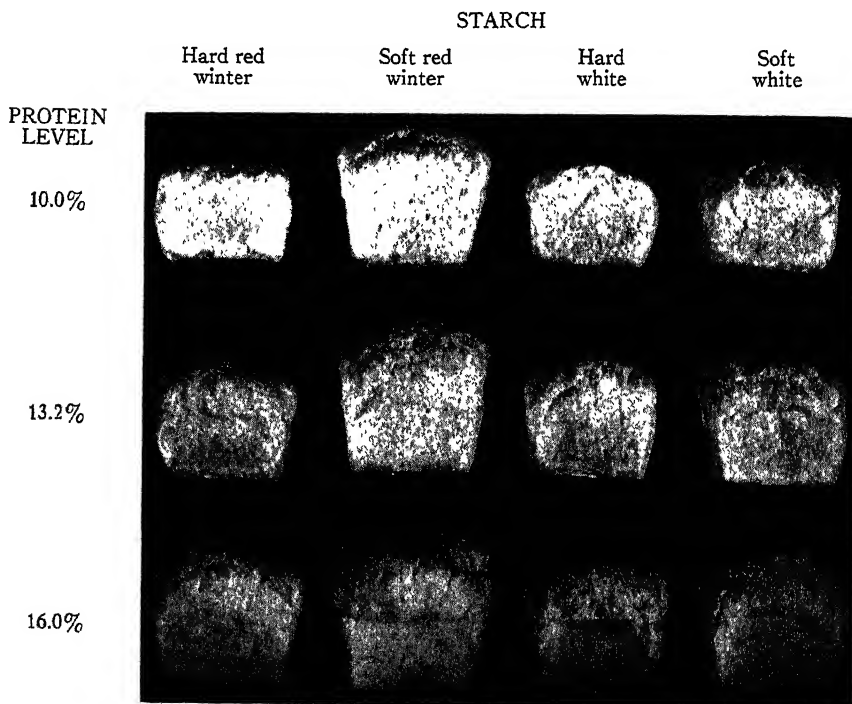
STARCH

Fig. 7.   Comparative loaves baked from blends of hard and soft winter and
white wheat starches with a common gluten substrate.

be obtained.   The phosphate-bromate baking method was employed,
with 7% sucrose.

The baking results showed marked differences between the protein
levels in terms of loaf volume, color, texture, and symmetry.   The
16.0% protein blends produced good loaves approximating, in many
instances, loaves baked from hard wheat flour of equivalent protein
content.   The absorption was raised in nearly every instance by
increasing the percentage of gluten in the blend.   Marked differences
in loaf volume are shown by blends made from the different starches.

The starch prepared from a soft red winter wheat variety, Wabash, produced the best loaf in the series. Superior results in terms of loaf volume were also yielded by starches from a hard red spring wheat, Thatcher, and the hard red winter varieties Nebred and Cheyenne. The latter varieties were grown in 1939. A sample of Chiefkan grown in 1938 was superior to the 1939 sample of Chiefkan.

A sample of emmer starch gave satisfactory results. The white-wheat starches baked into loaves which were below average in loaf
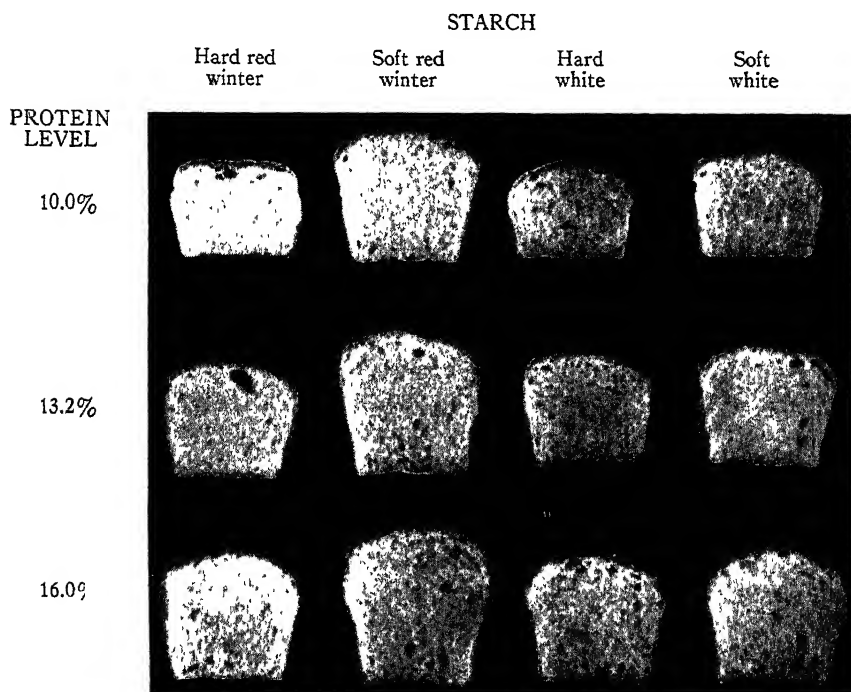
Fig. 8. Cut surfaces of loaves baked from blends of hard and soft winter and white wheat starches with a common gluten substrate.

volume. Turkey starch was also disappointing in its effect upon the loaf volume of the starch-gluten doughs, but the crumb color was satisfactory. Tenmarq starch, in like manner, was relatively low in loaf volume and symmetry at all protein levels.

It appears from the results that marked differences in those properties that influence baking quality are inherent in starches prepared as described from different wheat varieties. These differences may be related to injury to the starch granule during the operation of milling, with consequent effect upon the baking quality. The causes of such

dissimilarities are probably to a large degree heritable, but it is possible that environmental conditions are also involved. It is pointed out that in one instance where two samples of the same wheat grown in different years were examined considerable disparity in the loaves baked from the starch gluten blends was evident. Further investigations to answer these questions more fully are scheduled in the Department of Cereal Technology, North Dakota Experiment Station.

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THE ACTION OF THE AMYLASES OF TWO BARLEY VARIETIES ON THE BARLEY STARCHES

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(Read at the Annual Meeting, May 1940)

The malts made from the two varieties of barley, Oderbrucker C.I. No. 4666 (Wisconsin Pedigree 5–1) and Wisconsin Barbless C.I. No. 5105 (Wisconsin Pedigree 38), differ decidedly in certain characters determined by malt analysis (Dickson *et al.*, 1938). Wisconsin Barbless appears to be deficient in the various enzyme systems as determined by diastatic power, yield of extract, and the amount of nitrogen made soluble during the mashing process. The two varieties do not differ greatly in total quantity of starch, nitrogen, and pentosans in either barley or malt.

A great deal of work has been done on the amylases of barley and malt, the results of which have been reviewed by Hanes (1937). In these studies the substrates used have been primarily potato starch, although Stamberg and Bailey (1938, 1939) and Blish, Sandstedt, and Mecham (1937) have recently studied the action of the amylases of wheat on wheat starches. Very little information is available on the action of the enzymes on barley starches. Baker and Hulton (1938) found that the same hydrolytic products were obtained from the action of malt amylases on malt starch as on potato starch.

As a phase of the study of the factors constituting quality in malt, it seemed desirable to investigate the activity of the isolated alpha- and beta-amylases from two varieties of barley on substrates of their own starches.

Materials and Methods

The barleys used in this investigation, Oderbrucker and Wisconsin Barbless, were grown at Urbana, Illinois, in 1938. Malts were produced from these barleys in the experimental malting unit in the Regional Malting Laboratory as a part of the regional series for that year.

The methods described by van Klinkenberg as modified by Hanes (1935) were used in the isolation of the two amylases. The beta-amylase was extracted from the ungerminated barleys which had been pearled to remove the hulls, aleurone layer, and most of the germ tissue and then ground to a fine flour in a Wiley mill. A Sharples supercentri-

fuge was used to separate rapidly the precipitated enzyme from the alcohol solutions.

The alpha-amylases were prepared from the malts after the hulls had been removed and the remaining portion ground to a fine flour. A water suspension of the alcohol precipitate was heated to 70° C. for 15 minutes to inactivate the beta-amylase present, followed by precipitation with 65% alcohol, centrifuging, and drying. The Wigsman diffusion test and the Wohlgemuth method showed the enzymes to be relatively pure preparations.

The starches from the two varieties were isolated by a wet grinding process applied to the steeped, pearled barleys. The starch was passed through a 200-mesh sieve and the residue reground several times to increase the yield. The resulting starches were treated with 0.1% NaOH overnight at room temperature, washed several times by decantation, and finally collected and washed in a basket centrifuge. After a preliminary short drying in a vacuum oven, drying was completed in an air oven at 50°C. The yields of starch varied between 50% and 55% of the pearled barley.

Portions of each starch preparation were modified by the Lintner method with 7.5% hydrochloric acid at room temperature for 7 days, the acid being renewed every two days. In the following discussion these shall be referred to as modified starches. The analyses of the raw and modified starches are given in Table I.

TABLE I

CHARACTERISTICS OF THE STARCHES USED AS SUBSTRATES

Type of starch	Moisture	Total nitrogen	Total ash	Reducing power as theoretical maltose
	%	%	%	%
Raw—Oderbrucker	5.98	0.060	0.15	0.17
Soluble—Oderbrucker	7.30	0.035	0.30	3.56
Raw—Wisconsin Barbless	4.89	0.280	0.12	0.93
Soluble—Wisconsin Barbless	6.16	0.190	0.09	3.68
Soluble—Merck's Potato	11.92	0.014	0.26	2.28

The modified barley starches and Merck's soluble potato starch were used in 2% concentration, while the raw starches were used in 1% concentration. The untreated raw starches were triturated with several changes of distilled water, made to volume and suspended as well as possible by frequent shaking. The other starch solutions were prepared by adding the proper quantity of starch to boiling water and boiling for two minutes in order to disperse them. The solutions to be used with

beta-amylase were adjusted to pH 4.7 and those with alpha-amylase to pH 5.4 by appropriate acetic acid-sodium acetate mixtures.

All hydrolyses were carried out in cork-stoppered flasks in a water bath at 40°C. in the presence of a few drops of toluene, using 100 ml. of starch solution and 10 ml. of enzyme solution. In order to determine the reaction rates, a procedure similar to that used by Redfern and Johnston (1938) was used, aliquots being withdrawn at regular, short intervals until 30 minutes had elapsed from the time of addition of enzyme to the starch solution. The aliquots were immediately transferred to flasks containing alkaline ferricyanide reagent, and the reducing power determined by the method of Anderson and Sallans (1937). The reaction rates were determined from the slope of curves obtained by plotting milligrams of maltose produced against reaction time in minutes, and these were calculated for equal enzyme concentrations from the two varieties as milligrams of maltose produced per minute.

After five days the final reducing powers of the digests were determined and these were expressed as the percentage of the theoretical amount of maltose that could be obtained from the quantity of starch.

Presentation and Discussion of Data

The enzyme isolation procedures for the two varieties were made as nearly identical as possible in order to determine comparative yields. The respective yields of beta-amylase from Oderbrucker and Wisconsin Barbless barleys were 1.42 and 1.18 g. from 500 g. of barley flour, which was equivalent to 0.26% and 0.22% of the dry weight of barley flour. The yields of alpha-amylase from the malts from the same two barleys were 0.65 and 0.34 g. or 0.14% and 0.07% of the dry weight of the malts, respectively. The Oderbrucker variety yielded somewhat larger quantities of both enzymes than the Wisconsin Barbless.

Typical reaction-rate curves for alpha- and beta-amylase preparations from the Oderbrucker and Wisconsin Barbless barleys acting on the Oderbrucker soluble starch are shown in Figure 1. From an inspection of this figure, it is apparent that the activities of the alpha-amylases from the two varieties were not greatly different, while the beta-amylase from the Oderbrucker barley was more than twice as active as that from the Wisconsin Barbless. A summary of the reaction rates and hydrolysis limits of the four enzyme preparations upon the seven substrates is given in Table II. These values represent means of two or more duplicate runs. The data for reaction rates and hydrolysis limits, respectively, are illustrated by bar diagrams in Figures 2 and 3. No values are given for the reaction rates of either enzyme on the untreated raw starches
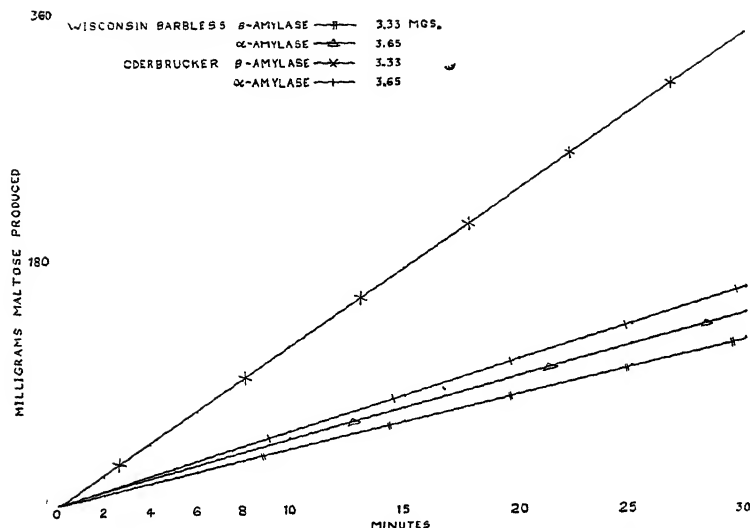
Fig. 1. Typical reaction rate curves for the action of the amylases on soluble Oderbrucker starch.

TABLE II

Summary of Reaction Rates and Hydrolysis Limits of the Alpha- and Beta-Amylases Acting Upon the Various Substrates (Means of Two or More Duplicate Runs)

Alpha-amylase 3.65 mg. per 110 ml.
Beta-amylase 3.33 mg. per 110 ml.

Variety and amylase	Activity	Type of substrate						
		Soluble potato	Soluble Oderbrucker	Soluble Wis. Barbless	Boiled raw Oderbrucker	Boiled raw Wis. Barbless	Raw Oderbrucker	Raw Wis. Barbless
Oderbrucker—Alpha	RR [1]	5.6	5.1	5.2	4.9	4.9	—	—
	HL [2]	55.0	53.4	56.9	51.3	50.4	2.5	15.3
Wis. Barbless—Alpha	RR	5.1	4.6	4.8	4.4	4.4	—	—
	HL	54.9	52.8	55.3	50.6	47.6	2.8	14.2
Oderbrucker—Beta	RR	11.0	11.6	9.7	7.3	5.7	—	—
	HL	61.8	62.8	53.9	48.5	53.6	1.2	2.9
Wis. Barbless—Beta	RR	3.9	4.0	3.7	2.0	1.9	—	—
	HL	60.6	63.3	56.4	45.8	50.4	1.3	3.1
Oderbrucker—Alpha + Beta	HL	—	—	—	—	—	4.5	18.4
Wis. Barbless—Alpha + Beta	HL		—	—	—	—	4.0	19.4

[1] RR indicates reaction rate in milligrams of maltose per minute.
[2] HL indicates hydrolysis limit after 120 hours as percentage of complete theoretical hydrolysis.

since the rates were so low that a satisfactory measurement could not be made.

From an inspection of Table II and Figure 2, it is evident that the alpha-amylase from the Wisconsin Barbless variety showed a lower activity on all substrates than that from the Oderbrucker, but the differences are not large. Considering the various substrates, the highest activity of the alpha-amylases was obtained on the soluble potato starch.
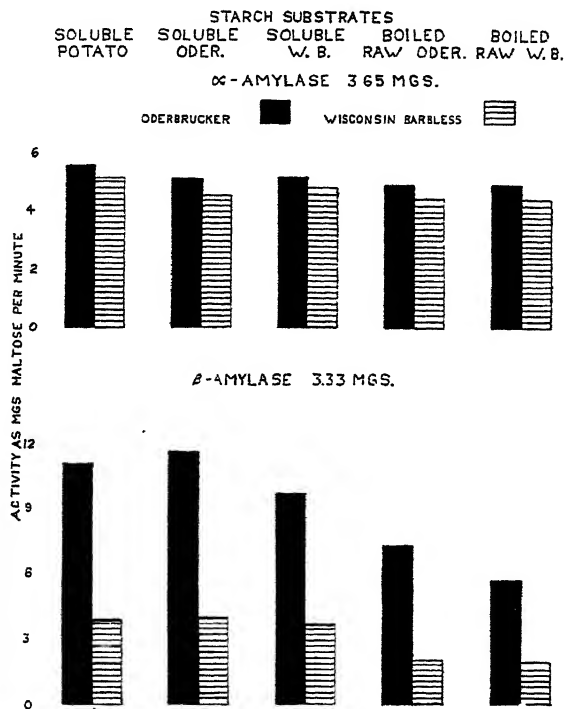
Fig. 2. Reaction rates, in milligrams of maltose per minute, of the alpha- and beta-amylases from the two varieties on five starch substrates.

The rate of action on the boiled raw barley starches was only slightly less than on the modified barley starches. Differences in the susceptibility of the starches from the two varieties were not readily apparent when only the activity of the alpha-amylases was considered, although there was a slight indication that the alpha-amylase from Wisconsin Barbless was somewhat more active on the modified starch from that variety than on the Oderbrucker starch.

The enormous difference in rate between the beta-amylases from the two varieties is shown in Table II and Figure 2. This difference was

much greater than would be anticipated from the diastatic powers of the two barleys based on the water extractions and the papain digestions. The values determined on the barleys were: for Oderbrucker, water extraction 66°L., papain digestion 125°L.; for Wisconsin Barbless, water extraction 56°L., papain digestion 122°L. The activity measurements were made at 40°C., while the diastatic powers were determined at the usual temperature of 20°C. It seems unlikely that the difference could be caused by a differential effect of temperature on the enzyme activities. Although as nearly identical procedures as possible were used for isolation, there is always the possibility of differences in solubility
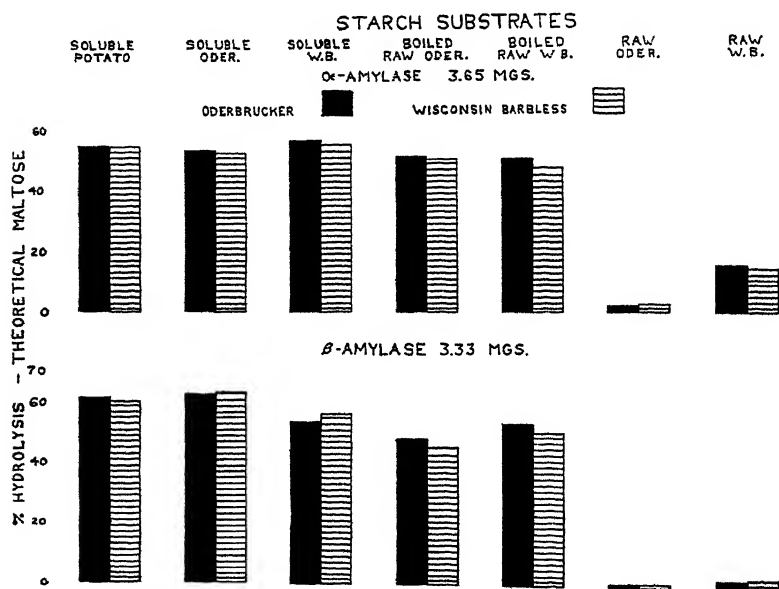
Fig. 3. Hydrolysis limits as percentage theoretical maltose after 120 hours, produced by the alpha- and beta-amylases from the two varieties acting upon seven substrates.

of the enzymes from the two barleys as well as the much debated question of the presence or absence of activators or inhibitors.

The Wisconsin Barbless soluble and boiled raw starches appeared to be more resistant to the action of the beta-amylase from the Oderbrucker variety than the corresponding Oderbrucker starches. The same trend was apparent with the Wisconsin Barbless beta-amylase, although the differences were small and probably not significant. As would be expected, the modified or soluble starches were much more susceptible to the action of beta-amylases than the boiled raw starches, in contrast to the action of the alpha-amylases where the differences were slight.

The hydrolysis limits in percentage of the theoretical maltose are given in Table II and Figure 3 for the four enzymes acting upon the seven substrates for 120 hours. Considering the alpha-amylases, the values varied between 50% and 57% hydrolysis for the modified and boiled starches and the variations due to source of enzyme and substrate were not great. A slightly higher limit was reached on the soluble Wisconsin Barbless starch than on the soluble Oderbrucker by the alpha-amylase from both varieties, which agrees with the results obtained on the reaction rates. Here again the hydrolysis limits obtained on the boiled raw starches were only slightly below those on the modified starches.

As noted earlier, the reaction rates of the Oderbrucker beta-amylase were much greater than the Wisconsin Barbless, but differences in hydrolysis limits were much smaller. In fact, on the modified starches from both varieties, the beta-amylase from Wisconsin Barbless hydrolyzed slightly more starch than that from Oderbrucker, but the difference was not significant. Considering the various substrates, the modified Wisconsin Barbless starch appeared to be somewhat more resistant to beta-amylase hydrolysis than the modified Oderbrucker starch. With the boiled raw starches from the two varieties the reverse seemed to be true. The hydrolysis limits obtained from the action of the beta-amylases on the soluble potato and soluble Oderbrucker barley starch were in the usual range reported in the literature (62%–67%) (Hanes, 1937). However, the final limits on the soluble Wisconsin Barbless starch and the boiled raw starches from both varieties were significantly reduced.

Several anomalies were evident. For example, the Wisconsin Barbless modified starch appeared to be more susceptible to the action of alpha-amylase and less susceptible to the action of beta-amylase than the corresponding Oderbrucker starch. There was some evidence that the reverse situation existed with the boiled raw starches. On the basis of the limits of hydrolysis by beta-amylase, the boiled raw Oderbrucker starch was appreciably more resistant to enzyme action than the modified Oderbrucker starch, but the corresponding Wisconsin Barbless starches did not show such a difference.

The action of the beta-amylase on suspended raw barley starch was very slight. The starch from Wisconsin Barbless was hydrolyzed more than twice as much as that from Oderbrucker. Likewise the Wisconsin Barbless starch was more than five times as susceptible to alpha-amylase. These facts, combined with the higher reducing power of this starch as shown in Table I, might indicate a larger percentage of broken granules in this sample as an explanation for its greater susceptibility. Microscopical examination did not show this to be the case, although there were

many more small granules in the Wisconsin Barbless starch than in the Oderbrucker.   The combined action of alpha- and beta-amylases on the raw starches was roughly equal to the sum of the individual actions of the two enzymes.   These values were obtained on suspensions that were not shaken continuously, and, as pointed out by Blish, Sandstedt, and Mecham (1937) accurate determinations of enzyme action are difficult under such conditions.

The combined action of the two amylases from Oderbrucker on the modified and boiled raw starches was investigated further.   In one case the alpha- and beta-amylases were allowed to act simultaneously; in the other, the alpha-amylase was allowed to act for 60 hours, then the beta-amylase was added and both allowed to act for an additional 60 hours. The results are given in Table III in which the bottom line represents

TABLE III

RESULTS ON THE COMBINED ACTION OF ODERBRUCKER ALPHA- AND BETA-AMYLASES
ON THE MODIFIED AND BOILED RAW STARCHES
Alpha-amylase 3.65 mg. per 110 ml.
Beta-amylase 3.33 mg. per 110 ml.

Combination of amylases	Activity	Type of substrate				
		Soluble potato	Soluble Oder-brucker	Soluble Wis. Barb-less	Boiled raw Oder-brucker	Boiled raw Wis. Barbless
Alpha- and beta-amylases simultaneously	RR [1]	13.4	15.5	14.2	13.8	12.2
	HL [2]	76.5	82.3	74.4	84.5	71.3
Alpha-amylase followed by beta-amylase	HL	78.7	70.8	65.0	82.2	75.8
Sum of individual alpha- and beta-amylases	RR	16.6	16.7	14.9	12.2	10.6

[1] RR indicates reaction rate in milligrams of maltose per minute.
[2] HL indicates hydrolysis limit after 120 hours as percentage of complete theoretical hydrolysis.

the sum of the individual reaction rates for the two enzymes as given in Table II.   As would be expected, the two enzymes acting together show a definite increase in activity over the individual action of either one. The combined action of the amylases on the soluble starches was not so great as the sum of the individual actions of alpha- and beta-amylase, but on the boiled raw starches the reverse was true.   This difference is probably explained by the fact that the boiled raw starches were almost as susceptible to alpha-amylase action as the soluble starches, but were considerably more resistant to beta-amylase.   However, when the enzymes acted together, the dextrin fragments from the action of alpha-amylase were rapidly broken down by the beta-amylase.   Alpha-amylase followed by beta-amylase gave a lower hydrolysis limit than the combined

action on the soluble barley starches, but on the boiled raw starches the differences were not great. In all cases where combinations of the two enzymes were used, the soluble and boiled raw Oderbrucker starches were more susceptible to hydrolysis than the corresponding Wisconsin Barbless starches.

A limited study was made on the influence of enzyme concentration on the hydrolysis limit, using a twofold increase of beta-amylase and two- and fourfold increases of alpha-amylase. The data are presented in Table IV. With the soluble potato and soluble Oderbrucker starch,

TABLE IV

The Influence of Concentration of Alpha- and Beta-Amylases on the Hydrolysis Limit After 120 Hours Obtained on the Various Substrates

Enzyme	Enzyme concentration, mg. per 100 ml.	Type of substrate				
		Soluble potato	Soluble Oder-brucker	Soluble Wis. Barbless	Boiled raw Oder-brucker	Boiled raw Wis. Barbless
		HL^1	HL^1	HL^1	HL^1	HL^1
Oderbrucker—Beta	3.33	61.8	62.8	53.9	48.5	53.6
	6.66	61.2	62.7	61.1	56.8	55.0
Wis. Barbless—Beta	3.33	60.6	63.3	56.4	45.8	50.4
	6.66	60.1	61.4	56.8	52.7	52.2
Oderbrucker—Alpha	3.65	55.0	53.4	56.9	51.3	50.4
	7.30	58.0	59.1	62.6	54.2	56.2
	14.60	64.7	71.7	75.4	63.2	64.9
Wis. Barbless—Alpha	3.65	54.9	52.8	55.3	50.6	47.6
	7.30	57.0	58.3	61.5	55.5	55.8
	14.60	63.4	67.3	72.7	69.6	62.8

[1] HL is the hydrolysis limit.

increasing the concentration of beta-amylase did not affect the hydrolysis limits and the values obtained agreed satisfactorily with those obtained by Hanes (1935). With the soluble Wisconsin Barbless starch, doubling the enzyme concentration increased the hydrolysis limit and gave a value which was in agreement with the other two soluble starches. In the case of the boiled raw starches, a greater enzyme concentration increased the hydrolysis limit, but the value was still appreciably below the soluble starch values.

It is seen that the hydrolysis limits obtained with alpha-amylase are dependent upon the enzyme concentration as shown by Hanes (1935). The limits of hydrolysis obtained were much higher than those generally obtained, although values of similar magnitude have been reported as summarized by Hanes (1937). This could be explained by contamina-

tion of the alpha-amylase by beta, although, as previously stated, both enzymes appeared to be relatively pure. Another possibility is that with the relatively long reaction time used, appreciable quantities of glucose may be formed which would increase the reducing power of the digest. These data substantiate those in Table II in showing that soluble Wisconsin Barbless starch is more susceptible to the action of alpha-amylase and less susceptible to the action of beta-amylase than that from Oderbrucker.

Several interesting differences in the starches as substrates have been indicated by this study, and further work is in progress in an attempt to explain them.

Summary

Preparations of alpha- and beta-amylases were made from Oderbrucker and Wisconsin Barbless barleys and malts. The starches were isolated from the same two barleys and portions modified in various ways.

The action of the amylases on the isolated starches was studied, reaction rates and final saccharification limits being determined.

Oderbrucker yielded 100% more alpha-amylase and 15% more beta-amylase than Wisconsin Barbless. The activities of the isolated alpha-amylases from the two varieties were about the same, but the beta-amylase from Oderbrucker was approximately 2½ times as active as that from Wisconsin Barbless.

Although the evidence is not entirely conclusive, there were indications that the modified and boiled raw Oderbrucker starches were more susceptible to beta-amylase degradation than the Wisconsin Barbless starches. However, the Wisconsin Barbless modified and raw starches were more susceptible to alpha-amylase action than the Oderbrucker starches.

Studies with combinations of the two enzymes, and the individual enzymes in different concentrations, essentially substantiated the above conclusions.

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FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH.[1] III. EFFECT OF PROTEIN CONTENT AND ABSORPTION ON THE PATTERN OF CURVES MADE ON THE RECORDING DOUGH MIXER

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Experience with a large number of curves made on the recording dough mixer has shown that the amounts of protein and of water used in mixing the dough influence the pattern, particularly the height of the curves. The illustrations presented in this paper will bring this out more fully. The wide variations that may occur in curves were shown by Swanson (1939). No attempt was made in that paper to relate these variations to protein content. Curves with little or no rise in the first part are generally from low-protein flours, while curves with a steep rise and good height are from high-protein flours. Variety also has an influence. Thus flours from Chiefkan and Clarkan, which belong to the Blackhull group, usually give curves which have a steeper rise and somewhat greater height than flours of the same protein content from Turkey and Tenmarq. Also, when the protein content of a hard red winter wheat is low the curves become similar to those obtained from soft red winter wheats (Larmour, Working, and Ofelt, 1939, 1940).

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[1] Contribution No. 72, Department of Milling Industry.

The purpose of this paper is to show how the curve pattern may be influenced by the protein content of the flours, whether this is the amount that naturally occurs in the flour or whether it has been modified by blending with starch or with a low-protein flour. It will also be shown that the percentage of absorption is an important factor especially in determining the height of the curves.
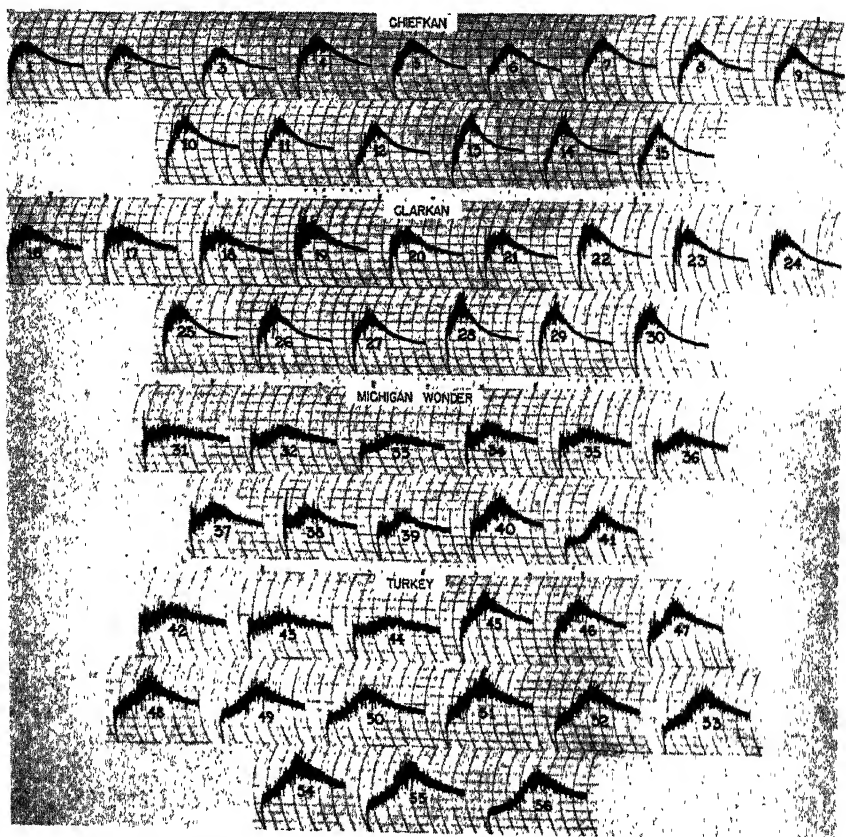
Fig. 1. Effects of variations in protein content and of absorption in flours from four varieties of wheat. (See Table I.)

Figure 1 shows the effects on curve patterns of variations in protein content and of absorption in flours from four varieties. These flours were milled from wheat varieties grown in co-operative experiments conducted in various parts of the state by A. L. Clapp of the Department of Agronomy. The wheat samples from various localities were blended so as to obtain samples at various protein levels.

In Table I are found the identification numbers for each of the curves, together with absorptions used in making them and also the protein contents of the flours used. The curves of each variety are arranged in groups of three, and the groups are arranged in ascending order of protein content. The first group to the left for each variety has the lowest protein, and the last group the highest protein. The middle curve in each group was made with the optimum absorption as determined on the flour-water dough. The first curve in each group was made with 2% less water and the third curve with 2% more water than the optimum. The amount of water needed to make a dough of

TABLE I

THE PROTEIN CONTENT AND ABSORPITON OF FLOURS OF FOUR
VARIETIES USED TO MAKE THE CURVES IN FIGURE 1

Variety	Sample No.	Protein	Absorptions			Curve Nos., Fig. 1		
		%	%	%	%			
Chiefkan	25042	9.9	58.0	60.0	62.0	1	2	3
	25043	11.2	60.5	62.5	64.5	4	5	6
	25044	12.7	63.5	65.5	67.5	7 ·	8	9
	25045	14.1	67.5	69.5	71.5	10	11	12
	25046	15.4	68.5	70.5	72.5	13	14	15 ·
Clarkan	25056	9.9	51.0	53.0	55.0	16	17	18
	25057	10.6	52.0	54.0	56.0	19	20	21
	25058	12.2	55.0	57.0	59.0	22	23	24
	25059	14.0	59.5	61.5	63.5	25	26	27
	25060	15.2.	62.0	64.0	66.0	28	29	30
Michigan Wonder	25079	9.7	52.0	54.0	56.0	31	32	33
	25080	10.6	53.5	55.5	57.5	34	35	36
	25081	12.5	55.5	57.5	59.5	37	38	39
	25082	14.3	63.0	—	67.0	40	—	41
Turkey	25096	9.5	55.0	57.0	59.0	42	43	44
	25097	10.8	62.0	64.0	66.0	45	46	47
	25098	12.7	62.0	64.0	66.0	48	49	50
	25099	13.8	63.5	65.5	67.5	51	52	53
	25100	15.6	67.5	69.5	71.5	54	55	56

optimum consistency was determined by mixing flour and water in a micro mixer and "feeling" the dough. This method leaves much to be desired, since the results depend so much on the experience and judgment of the operator. However, it was the only practical method available for these experiments.

The curves in Figure 1 show that variations of 2% above or below the optimum absorption do not change the general "varietal" pattern except in height. An absorption below optimum will give a greater height than an absorption above the optimum. With some varieties such as Turkey an absorption above the optimum will have a tendency toward

production of a concave upslope. This tendency is not shown in the curves from Chiefkan and Clarkan, in which the upslope is convex. This concave upslope is also shown when the wheat has been damaged by high moisture (Swanson, 1941).

Thus the amount or percentage of absorption will influence the height, and in some flours the character of the upslope, but will not within reasonable limits modify the main pattern of the curves. The protein content is the more potent factor in affecting the height of the curves.

The varietal factors have the greatest influence on the curve pattern (Swanson, 1938). Thus in Figure 1 it is evident that Chiefkan has patterns very different from the others. As will be shown later, the patterns due to varietal characteristics are less pronounced in curves from low- than in curves from high-protein flours. That the protein content influences the height has also been shown by Larmour, Working, and Ofelt (1939). The height, however, is much less influenced by protein content in Chiefkan and Clarkan than in Michigan Wonder and Turkey.

Effect of Reducing the Protein Content to 10% by Adding Starch

The same flours of the four varieties used for making the curves shown in Figure 1 were diluted with wheat starch [2] so that all the mixtures had 10% protein. The curves obtained from Chiefkan, Clarkan, Michigan Wonder, and Turkey are shown in rows 1, 2, 3, and 4 respectively of Figure 2.

The legend for Figure 2 identifies each curve by number and gives the protein percentages of the original flours and the absorptions used. The first curve in each one of the four rows was made from the un-diluted flour, since the protein content was a little below 10% as shown in Table I. For Chiefkan and Clarkan, in which the protein was very near 10%, the first curves show very little difference from those that were diluted. The curves made from the undiluted flour from Michigan Wonder and Turkey show notable differences from those that were made from the flours diluted to 10%.

In the fifth row of Figure 2 are shown curves that bring out the contrast between those made from Tenmarq (protein 12.8%), Turkey (protein 13.1%), and Chiefkan (protein 12.8%), both on the original flours and after dilution to 10% protein.

A comparison of curves in Figures 1 and 2 will show that while protein content is the greatest factor in influencing the pattern of the curves, the curve differences within a variety largely disappear when

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[2] This was furnished by the Huron Milling Company, Huron, Michigan.

the protein content is reduced to the same level. Thus the curves from the flours in the range of 14% to 15% protein are similar to those that had been diluted from only a little above 10%. The main effect of dilution with starch is to reduce the height, but the varietal charac-

Fig. 2. Protein reduced to 10% by addition of starch.

CHIEFKAN						
No. of curve	1	2	3	4	5	
Original protein, %	9.9	11.2	12.7	14.1	15.4	
Absorptions, %	62	64	65	66	67	
CLARKAN						
No. of curve	6	7	8	9	10	
Original protein, %	9.9	10.6	12.2	14.0	15.2	
Absorptions, %	62	62	62	62	61	
MICHIGAN WONDER						
No. of curve	11	12	13	14		
Original protein, %	9.7	10.6	12.5	14.3		
Absorptions, %	60	61	62	61		
TURKEY						
No. of curve	15	16	17	18	19	
Original protein, %	9.5	10.8	12.7	13.8	15.6	
Absorptions, %	64	67	67	68	68	

	TENMARQ		TURKEY		CHIEFKAN	
No. of curve	20	21	22	23	24	25
Original protein, %	12.8	—	13.1	—	12.8	—
Absorptions, %	68	68	64	66	62	65

teristics still persist. Thus if the protein content is made constant, all the curves from the flours of any one variety that differ in protein will be very similar. Whether these flours diluted with starch would have the same baking characteristics as 10% protein flours was not determined.

Effect of Progressive Dilution with Starch

Flours from Turkey (protein 13.1%), Tenmarq (protein 12.8%), and Chiefkan (protein 12.8%) were diluted progressively with the wheat starch used and also with ordinary edible corn starch. The proportions of flour and starch, and the calculated percentages of protein of the mixtures, are presented in Table II and the curves obtained from these mixtures are shown in Figure 3.

TABLE II

PROPORTIONS OF FLOUR AND STARCH MIXTURES AND PROTEIN PERCENTAGES

Proportions		Calculated protein content		
Flour	Starch	Turkey	Tenmarq	Chiefkan
g.	g.	%	%	%
35	0	13.10	12.80	12.80
30	5	11.19	10.98	10.97
25	10	9.32	9.15	9.14
20	15	7.45	7.32	7.31
15	20	5.59	5.49	5.48
10	25	3.73	3.66	3.66

The upper three rows are from dilution with wheat starch, and the lower three rows are from dilution with corn starch. The curves in Figure 3 show a progressive decrease in height with increasing starch ratios. The variety characteristics persist until the flour-starch ratio is about 20–15. The effect of corn starch is very different from that of wheat starch in the larger starch ratios. In these, the elastic characteristics have disappeared and the flour-starch-water mixture behaves more as a plastic mass. The same may be said of Chiefkan for the two larger wheat-starch ratios. The Turkey and Tenmarq mixtures with the larger amounts of wheat starch produce curves similar to those obtained from very soft, low-protein flours. The curves with the larger starch ratios make it appear that the flours of Tenmarq and Turkey in comparison with Chiefkan have longer gluten strands (Swanson, 1925, 1938), which maintain the dough characteristics when larger amounts of starch are present. In Chiefkan they seem to be shorter, and hence the plastic properties become evident with the smaller starch-flour ratios. There were some variations in absorptions of the different ratios of flour and starch, but these were not sufficient to have any material influence on the general patterns of the curves shown in Figure 3.

Effect of Blending a Low-Protein Flour with High-Protein Flours

A soft wheat flour of 8.3% protein was blended in various proportions with flours of Turkey, Tenmarq, and Chiefkan. The proportions
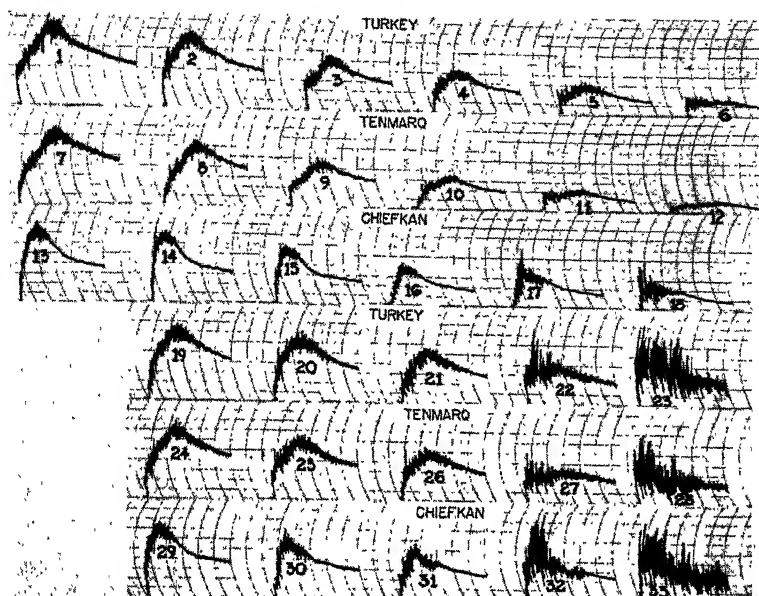
Fig. 3. Curves showing effect of progressive dilutions with starch.

Ratio of Flour to Starch

	35–0	30–5	25–10	20–15	15–20	10–25
		WHEAT STARCH (*Curve Numbers*)				
Turkey	1	2	3	4	5	6
Tenmarq	7	8	9	10	11	12
Chiefkan	13	14	15	16	17	18
		CORN STARCH (*Curve Numbers*)				
Turkey		19	20	21	22	23
Tenmarq		24	25	26	27	28
Chiefkan		29	30	31	32	33

of the blends, the calculated protein percentages of the blends, and the absorptions of the blends as well as the flours used in blending are given in Table III. The curves obtained on these blends are given in Figure 4. The first curve in each row is from all low-protein flour, and the last from all high-protein flour. The four intermediate are from the blends as indicated in Table III.

The curve from the low-protein flour used in blending reaches the maximum height in about a half minute. The upslope and the downslope are so short that they are almost obscured. At the end of the short downslope the curve becomes horizontal with a slow narrowing. The upward rise of the curves from the blends starts with the smaller proportion of the hard-wheat flours and becomes higher with the increasing amounts of these flours. The percentages for absorption increase, as would be expected, with the larger amounts of the high-protein

flours, the increases being greatest in Chiefkan and least in Turkey. The gradual change in the characteristics of these curves with increasing amounts of the high-protein flours indicates that the absorption percentages were suitable to these mixtures.

TABLE III
BLENDS OF LOW-PROTEIN FLOUR WITH HIGH-PROTEIN FLOURS

Proportions in blend		Calculated percentages protein in blends with			Absorptions of blends with		
Low protein	High protein	Turkey	Tenmarq	Chiefkan	Turkey	Tenmarq	Chiefkan
		%	%	%	%	%	%
100	0	8.3[1]	8.3[1]	8.3[1]	57[1]	57[1]	57[1]
80	20	9.3	9.2	9.2	58	59	59
60	40	10.2	10.1	10.1	60	61	62
40	60	11.2	11.0	11.0	61	63	64
20	80	12.1	11.9	11.9	62	64	66
0	100	13.2	13.0	13.1	63	66	68

[1] Protein percent and absorption of the soft-wheat flour used in mixing with the high-protein flours.
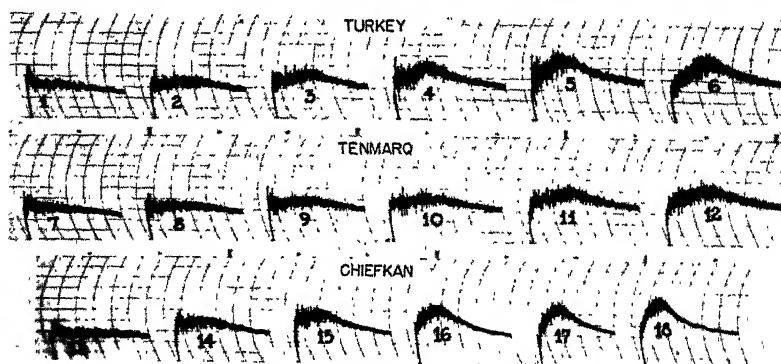
Fig. 4.   Curves showing effect of blending a low-protein flour with high-protein flours.

	TURKEY					
No. of curve	1	2	3	4	5	6
Protein, %	8.3	9.3	10.2	11.2	12.1	13.2
	TENMARQ					
No. of curve	7	8	9	10	11	12
Protein, %	8.3	9.2	10.1	11.0	11.9	13.0
	CHIEFKAN					
No. of curve	13	14	15	16	17	18
Protein, %	8.3	9.2	10.1	11.0	11.9	13.1

Reducing High-Protein Flours to 11% by Blending with a 8.3% Protein Flour

The same low-protein soft wheat flour used in the preceding experiment was used to blend with several high-protein flours so that the mix-

tures were 11% protein. The protein and the absorption percentages are given in Table IV. The curves obtained on the original flours as well as the blends are given in Figure 5. In each pair of rows, the curves in the upper row are from the original flours and in the lower row from the 11% protein blend. The first two curves in the lowest row of Figure 5 are from the original flours and the last two from the 11% blend.

TABLE IV

EFFECT UPON ABSORPTIONS WHEN FLOURS WERE BLENDED WITH LOW-PROTEIN FLOUR TO A UNIFORM 11% PROTEIN CONTENT

Variety	Protein original	Absorption	
		Original	Blend
	%	%	%
Turkey	12.7	64.0	61
	13.2	63.0	60
	13.8	65.5	61
	15.6	69.5	62
Chiefkan	12.7	65.5	62
	13.1	68.0	63
	14.1	69.5	63
	15.4	72.5	63
Clarkan	12.2	57	57
	14.1	62	59
	15.4	64	60
Michigan Wonder	12.5	58	58
	14.8	65	60

The effects of diluting high-protein flours with a low-protein were similar to what was obtained by diluting with starch as shown in Figure 2. That is, all the curves from the blends are similar or very nearly so whether the dilution was from the higher- or the lower-protein flour. The figures for absorption were also nearly the same.

Discussion

The amount of water in a flour-water dough is about 40%–45% of the weight of the dough (Swanson, 1938). The greater part of this exists as adsorbed water on the starch and gluten, each probably holding equal amounts in a dough made from a flour of average or about 11% protein (Alsberg, 1927). It is assumed that in such a flour the protein holds as much adsorbed water as the starch, although this is present in six to seven times the amount of gluten (Swanson, 1938).

This assumption may explain why the absorptions are larger with an increase in protein. This adsorbed water is held on the surfaces of the starch and gluten material in varying degrees of freedom, depending on
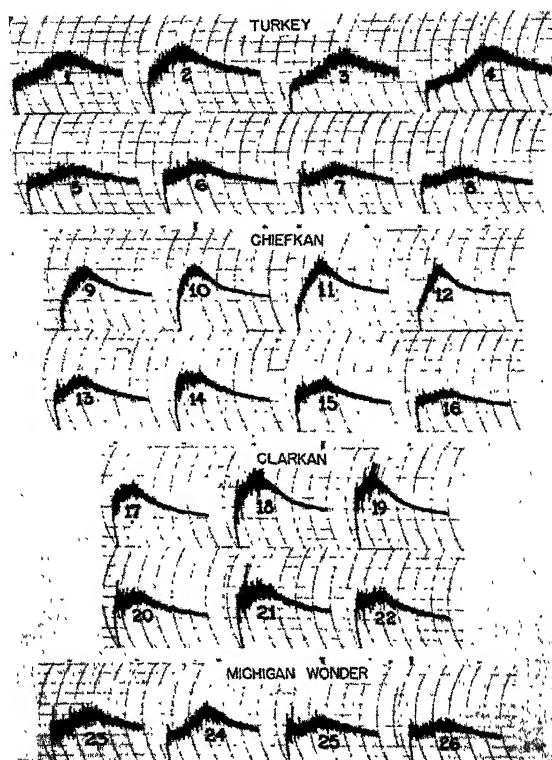
Fig. 5. Curves showing effect of reducing high-protein flours to 11% by blending with 8.3% flour.

TURKEY

		Original Protein				Reduced to 11%		
No. of curve	1	2	3	4	5	6	7	8
	12.7%	13.2%	13.8%	15.6%				

CHIEFKAN

		Original Protein				Reduced to 11%		
No. of curve	9	10	11	12	13	14	15	16
	12.7%	13.1%	14.1%	15.4%				

CLARKAN

		Original Protein			Reduced to 11%	
No. of curve	17	18	19	20	21	22
	12.2%	14.1%	15.4%			

MICHIGAN WONDER

		Original Protein	Reduced to 11%	
No. of curve	23	24	25	26
	12.5%	14.8%		

the distance from the adsorbing surface. The layers of water molecules next to the adsorbing surface are bound like the molecules in the starch and gluten material, since there is a possible chemical combination (Hauser, 1939). It is not known how deep this film of bound water is, but no doubt a considerable portion of the water in the dough is in this condition.

Such water does not function in giving the dough its characteristic properties. In the outer layers of water molecules away from the adsorbing surface, the freedom to move among others increases more and more until it is as great as in liquid water. That a portion of the water in the dough has this freedom is known from the fact that the vapor pressure of dough is equal to that of liquid water. It is the water which has this degree of freedom that contributes most to the characteristic properties of dough. The boundary line between free and bound water is not sharp (Hauser, 1939). Next to the surface the water molecules are held as though in chemical combination, but farther away from the surface there is a transition zone between the free and the bound water. In the inner part of this zone the molecules are more bound, and in the outer part they gradually approach the freedom that exists in liquid water. These partially bound water molecules in the outer layers may contribute something to the consistency of dough.

How much water is in the more free condition and how much is adsorbed or bound is not known, and as has been intimated the boundary line is not sharp. Calculations can be made to show that about one-third of the water in the dough is in the free condition (Swanson, 1938). Figures given by Alsberg (1927) indicate that gluten and starch each hold two-fifths of the total water and that one-fifth is free. This assumption, however, was based on work with soft wheat. Skovholt and Bailey (1935) used the term "bound water" in the sense that it is the difference between the total and free water present. They found the average bound-water value as a result of their determinations to be 51.4% of the total water present. This would indicate a much larger amount of water in the free condition than is often supposed. Vail and Bailey (1940) state that the average calculated bound water was found to be 35.5%. When water is added to make the dough, the bound-water demand would be satisfied first. This demand is probably fairly constant for any one flour. Any variation in absorption would have a multiple effect on the amount of free water, or the water that has most to do with the consistency of dough. It is for this reason that small deviations from optimum absorptions have an important effect on dough consistency.

The gluten in dough developed by mixing seems to exist as a three-dimensional network (Swanson, 1925). When water first comes into contact with the protein to form gluten, this substance is arranged in a heterogeneous pattern. The mixing, when done in the pull-folding type of machine (the principle of most commercial mixers), orients these gluten strands into a more or less parallel system. The maximum resistance of dough to mechanical action apparently occurs when this parallel arrangement has been attained. The higher the protein and consequently the larger the quantity of the gluten strands, the greater the resistance, and hence, the higher the top of the curve. The dilution with starch or with a low-protein flour will attenuate this gluten network, causing a decrease in resistance, and hence the top of the curve will be lower.

Summary

The curves and data presented in this paper indicate the following:

The main characteristics of the curves obtained on the recording dough mixer with flours from sound wheats are determined by variety. That is, qualities inherent in a variety give curves of a certain pattern. Some varieties give patterns very similar to those of other varieties.

Within any variety the curves are influenced by the protein content and by absorption. A 2% variation in absorption will notably affect the height of the curve, but the main pattern due to varietal characteristics is not affected.

The protein content is the most potent factor within a variety. Given a reasonably correct absorption the height of the curve will be greater with increasing protein content. The distinctive varietal characteristics are more pronounced in the curves from medium- and high-protein flours than from low-protein flours. When high-protein flours are diluted with starch or a very low-protein flour so that the protein contents of the blends are equal, the patterns of the curves from the blends will be the same within a given variety, but the curves of the blends of one variety will be different from those of another variety, provided of course that the original flours from these varieties gave different curve patterns.

Theories are given that explain why variations in absorptions and protein contents influence the patterns of the curves.

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QUANTITY OF DOUGH IN RELATION TO THE USE OF THE FARINOGRAPH [1]

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The farinograph has proved to be a very useful instrument in certain types of studies on wheat and flour quality and during the past ten years numerous papers have been published which have been based on farinograms. The characteristics of the curves which have been measured are primarily: (1) the height of the curve (oftentimes uniform at the maximum), (2) the time to reach maximum plasticity, (3) the slope of the curve from the peak to some arbitrary end as measured by areas or other means, (4) the width of the curve at various stages. Some authors have subjected the data thus obtained to statistical analyses in relation to baking-test data.

Judging from the details given in various publications containing farinograph data, the precaution of keeping the quantity of dough constant has apparently been overlooked except in the instance of Near and Sullivan (1935), who used a constant quantity of 480 g. of dough in their study of flour absorption. Others, and particularly Skovholt and Bailey (1932), have shown the necessity of maintaining the temperature constant for accurate results with the farinograph. This report will indicate that the quantity of dough is also a factor to consider. A comparison of results with the large 300-g. bowl and the small 50-g. bowl was also made, with identical conditions and materials.

Experimental

Three flours were used with protein contents, at 15% moisture, of 14.83%, 10.87%, and 8.12%, respectively. Flour-water doughs with 2% salt were mixed at 30° and for 30 minutes. The ratios of each ingredient were calculated to give a total amount of 480 g. of dough when the large bowl was used and one-sixth of that quantity or 80 g. for the small bowl. When other amounts of dough were used the same ratios of the ingredients were also maintained for each flour.
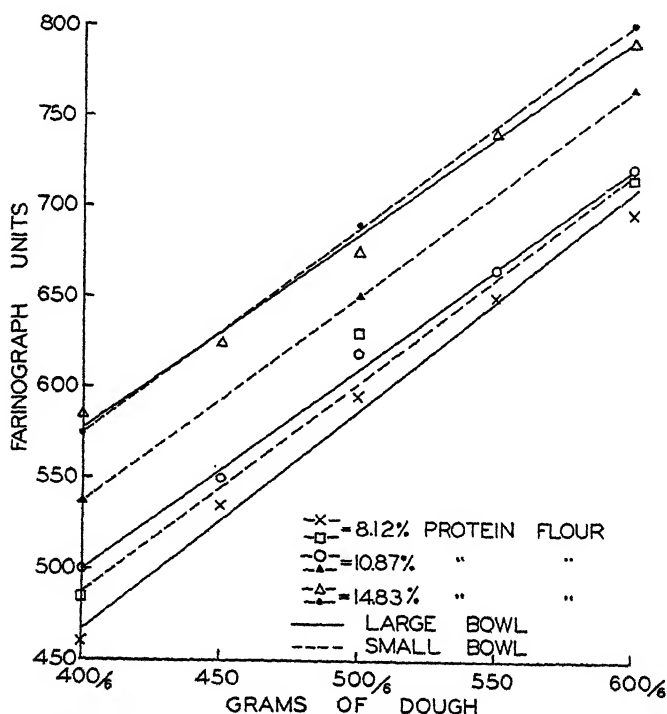
Fig. 1. The effect of the quantity of dough in the large and the small farinograph bowls on the height of the point of maximum plasticity in farinograph units. As the quantity of dough was varied the ratio of flour, total water, and salt was maintained constant for each flour.

Effect of the amount of dough.—Figure 1 shows the results of varying the amount of dough from 400 to 600 g. in the large bowl and from 66.6 g. (400/6) to 100 g. (600/6) in the small bowl. Farinograph units (F.U.) at the point of maximum plasticity are plotted against the quantity of dough. The slopes of the lines are approximately the same for all three flours and with both bowls.

Taking the average value of all the lines shown in Figure 1, the change in height of the farinograph curves per unit weight of the

doughs is 0.98 F.U. per gram of dough in the large bowl or per 0.167 g. in the small bowl. For all practical purposes it can be taken as one unit per gram or one unit per 0.167 g. in the large bowl and small bowl respectively.

Referring to the results obtained with the large bowl, the variation in the quantity of dough is usually not as large as from 400 to 600 g., but the variations generally are of significant proportions in ordinary work and can range from 450 to 550 g., thus causing a difference in height of the curve of up to 100 units. For instance, if a low-protein flour is mixed to give a flour-water dough with a maximum plasticity of 500 F.U., the absorption is about 50% on the fully corrected 15% moisture basis, thus giving a total quantity of dough of 450 g., which is perhaps the smallest quantity of dough generally encountered. The literature contains absorption figures of up to 70%, and sometimes higher, for high-protein flours. Such high absorption values are perhaps on the " as is " basis, but would give 510 g. of dough. Thus a difference of 60 g. is possible on flour-water doughs. When other ingredients are added such as salt, milk solids, sugar, malt, shortening, and others, the total quantity of dough can well be 550 g. or more. Since the height of the curve has been given so much significance, the necessity of controlling the quantity of dough becomes quite apparent for exact work and especially in research. The routine analyst might find it too cumbersome to adjust the ratios of ingredients to give a constant quantity of dough, although it appears desirable.

The quantity of dough used by Near and Sullivan (1935), 480 g. in the large bowl, appears to be optimum, since with larger quantities all of the dough is not agitated with each turn of the blades and this apparently accounts for the fluctuations in the 600 g. dough curves (or 600/6 dough curve) in Figure 2. These fluctuations were observed to a lesser extent in some 550-g. doughs.

The observations made with the large bowl also apply to the small bowl except that the quantity of dough considered is one-sixth of that used in the large bowl. In using the small bowl, it is apparent, of course, that any errors in weighing and measuring are six times as significant as when the large bowl is used.

Comparison of the large and the small bowls.—Figure 2 shows farinograms made from two flours with 400, 500, and 600 g. of dough in the large bowl and one-sixth of these quantities in the small bowl in the case of one flour. Each set of doughs was made to include exactly the same ratio of flour, water, and salt, and differed only as to the quantity of dough in the mixing bowl. The variation in height of the curve is quite evident.
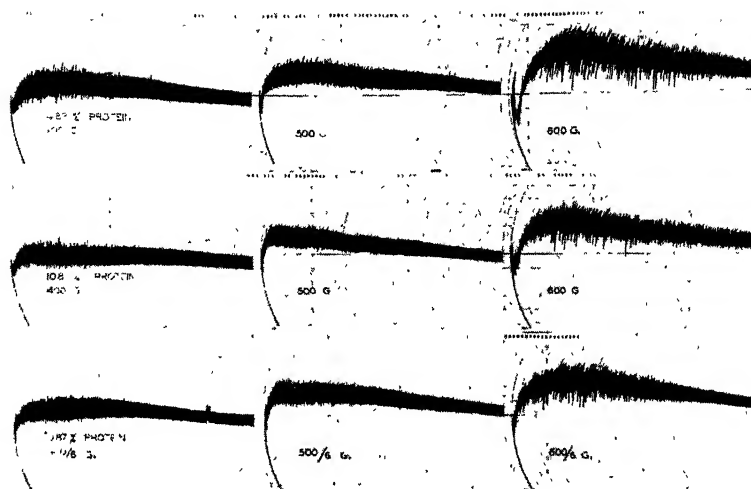
Fig. 2. Farinograms made with different quantities of dough prepared with the same ratio of flour, water, and salt. The two upper rows were made with the large bowl, and the lower with the small bowl. The two lower rows were made with the same kind and ratios of ingredients.

A comparison of the lower two rows of farinograms in Figure 2, made with the 10.87% protein flour, shows that curves obtained with the large bowl appear much like those obtained with the small bowl. Yet, a careful analysis of the curves made with the large and the small bowls reveals some variations in results. The measurements of curves made with two flours, and each flour at two absorption levels, are given in Table I. It is quite clear that the curves obatined with the small

TABLE I

COMPARISON OF THE LARGE AND THE SMALL FARINOGRAPH BOWLS

Flour protein content	Bowl	Absorption used	Time to maximum plasticity	Difference	Maximum plasticity	Difference	Plasticity at 30 min. mixing	Difference	Difference in plasticity at maximum and at 30 minutes
%		%	min.	min.	F.U.	F.U.	F.U.	F.U.	F.U.
14.83	Small	56	6.5	− 1	700	55	585	60	115
"	Large	"	5.5	—	645	—	525	—	120
"	Small	61	14	+ 2	510	30	445	0	65
"	Large	"	16	—	480	—	445	—	35
10.87	Small	50	2	+ 2	710 ·	50	570	30	140
"	Large	"	4	—	660	—	540	—	120
"	Small	55	12.5	0	485	40	410	45	75
"	Large	"	12.5	—	445	—	365	—	80

bowl were not exactly identical with those obtained with the large bowl. although the ingredients were in the same ratios and the quantities of dough used were 480 and 80 g. for the large and the small bowls, respectively.

The farinograph records, as consistency units, the work required to turn the blades through the dough in the bowl. If more dough is used, even of the same consistency as maintained by a constant ratio of ingredients, more work will be recorded as consistency units. It is thus apparent that variations in weight of dough will cause corresponding variations in consistency units that have nothing to do with consistency, but rather with work input. In ordinary work these differences may not be important but when measurements are made of farinograms for statistical interpretations such differences might be significant.

The bowls used in this study were of the newer type with adjustments, and it has been pointed out to the authors that perhaps the adjustments were not properly made. However, the explicit directions made by the manufacturer were rigidly followed in using both bowls. Furthermore, if it were a question of adjustment, the differences between the small and the large bowl should probably be constant for various doughs, but this was not the case.

Mixing bowl cover.—In connection with this discussion it might be well to indicate the need of covering the mixing bowl while doughs are being mixed in it. In this laboratory a heavy piece of plate glass has been used as a cover and the heavy condensation of moisture on this during the 30-minute mixing period suggests an appreciable loss in moisture unless a cover is used. Such loss of moisture will affect the plasticity of the dough. This precaution of using a cover is of course quite imperative when the humidity of the laboratory atmosphere is low.

The need of using a constant quantity of dough for accurate work might also be true in case of other types of recording dough mixers.

Summary

Plasticity of the dough as recorded by the farinograph is an almost linear function of its weight when the ratio of ingredients is kept constant. It is found to increase very nearly one farinograph unit per gram of increase in the weight of dough.

Constant quantity of dough is shown to be essential in the accurate use of the farinograph, just as it has previously been shown that constant temperature is essential to accuracy.

Optimum charge of ingredients in the large bowl appears to be 480 g. and one-sixth of that quantity, or 80 g., in the small bowl.

Curves made on the same flour with a constant ratio of ingredients at three weight levels with both bowls were not identical when comparable precise measurements were made of them.

Covers for the bowls are recommended to prevent loss of moisture from the doughs during a test.

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SOME STUDIES ON FLOUR ABSORPTION [1]

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(Read at the Annual Meeting, May 1940)

Many attempts have been made to standardize a technique for the determination of the value generally referred to as flour absorption, and Near and Sullivan (1935) and Bailey (1940) discuss the various instruments and methods that have been used. The absorption of a flour can be considered as the amount of liquid, accurately calculated and expressed on a standard flour moisture basis, that is required to give a dough with proper handling and machining properties and that will produce the best final baked product. The value varies with formula and baking method as well as with the procedure used. Thus in view of the many types of bakery products and baking procedures, it naturally becomes difficult to express the absorption of a flour as a single value. Yet a single relative value of the water-absorbing capacity of a flour, based on a standard formula and technique, becomes quite useful even when other formulas and methods are used, and such procedures have been generally adopted.

This is a study of doughs from several flours made at different absorption levels as compared by the use of the farinograph, the pressure plastometer described by Stamberg and Bailey (1940), and by various baking tests.

Calculation of Absorption

Before presenting the experimental data it appears pertinent to discuss the methods of calculating absorption. It seems that three different methods have been used in establishing absorption values, although only one can be considered correct, and unfortunately in many publications the method used has not been stated. The three methods and an example of calculating each are listed:

1. The fully corrected method, basis 15% moisture. Let the flour contain 12% moisture. Then 96.6 g. contains 85 g. of dry matter. One must add 3.4 g. of water to make the total equal 100 g. Whatever water over and above this amount that is used in making the dough is all that can be called the absorption of 100 g. of flour, basis 15% moisture. If 58 g. of water is used, beyond the 3.4 g. already specified for correction of flour weight, then 58 is the percentage of absorption. For any moisture content of the flour the absorption value remains a constant.

2. The dry matter corrected method, basis 15% moisture. Let the flour contain 12% moisture. Then, as before, 96.6 g. of flour contains 85 g. of dry matter. To make up a dough, as before, $3.4 + 58 = 61.4$ g. of water is used, and 61.4 is given as the percentage of absorption.

3. The "as is" basis. Again, let the flour contain 12% moisture. If a dough of the same plasticity were made up, using 100 g. of flour, the quantity of water required would be $(61.4 \div 96.6) \times 100 = 63.7$ g. This is reported as the percentage absorption on the "as is" basis. It is readily seen that as the flour loses moisture, its "as is" absorption increases, leading to a fictitious value for the flour.

TABLE I

FLOUR ABSORPTION BY DIFFERENT METHODS OF CALCULATION

Flour	Moisture content of flour	Absorption, 15% moisture basis, fully corrected	Absorption, 15% moisture basis, dry matter corrected	Absorption on "as is" basis
	%	%	%	%
1	8.4	56.0	63.3	68.3
2	10.1	56.0	61.5	65.5
3	11.0	56.0	60.5	63.4
4	12.0	56.0	59.4	61.5
5	14.0	56.0	57.1	57.8

The difference in values obtained by these three methods depends of course on the moisture content of the flour as can be seen by the calculated hypothetical values in Table I. When the moisture content of the flour is low, or 8.4%, the absorption values range from 56.0% to 68.3%, or a difference of 12.3%. When the moisture content of the flour

approaches the moisture basis used the difference is smaller, as at 14% moisture the difference is only 1.8% in the absorption values.

The apparent use of all three methods perhaps accounts for the rather wide range of absorption values occurring in the literature, and it appears quite desirable to use the fully corrected method since such data could then be used to find the correct ratio of liquid to dry matter.

In this laboratory a graph is used to find the correct weight of flour to use in order to provide 85 g. of dry material at various moisture levels from 0% to 15%. It is not a straight line relationship as often
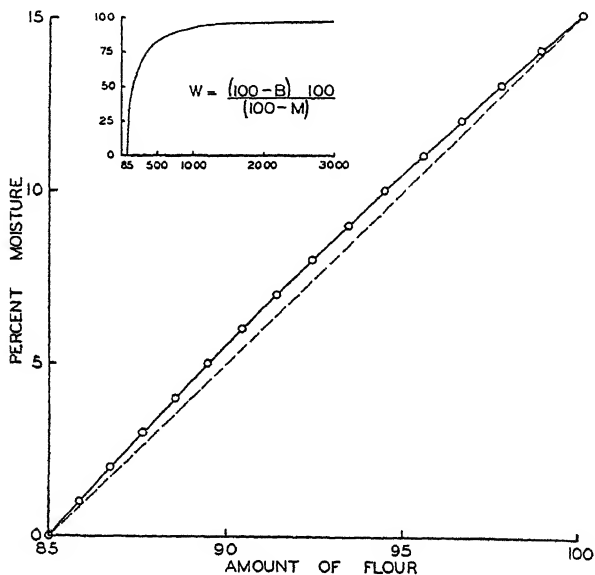
Fig. 1. The continuous line of the large graph passes through plotted values of flour weights containing 85 g. of dry matter for percentage moisture content, ranging from 0% to 15%. The broken line indicates the departure of the graph from that of a straight line. The small graph plotted in the same way, with ordinates as moisture content of flour, abscissas as grams of flour, but over a range of moisture content of flour from 0% to near 100%, shows the hyperbolic nature of the curve.

believed, but slightly curvilinear as shown in Figure 1, and it is part of a hyperbola of the general equation:

$$W = \frac{(100 - B)\,100}{100 - M},$$

where W = the weight of flour to use to provide a constant weight of dry material.

B = flour moisture basis used. The large graph in Figure 1 is plotted for the 15% moisture basis with a range in moisture content of flour from 0% to 15%.

M = percentage moisture in the flour.

The same formula can be used, of course, to obtain the curve for any other moisture basis. All absorption values included in the subsequent paragraphs are on the 15% moisture basis and by the fully corrected method.

Farinograph and Plastometer Tests

A series of twelve commercially milled flours ranging in protein content from 7.26% to 15.50% at the 15% moisture basis were used. Flour-water doughs containing 2% salt were mixed in the farinograph at 30° C. and the percentage absorption necessary to give a maximum plasticity of 500 F.U. was determined. The ratio of ingredients was adjusted to provide 480 g. of dough in accordance with the recommenda-
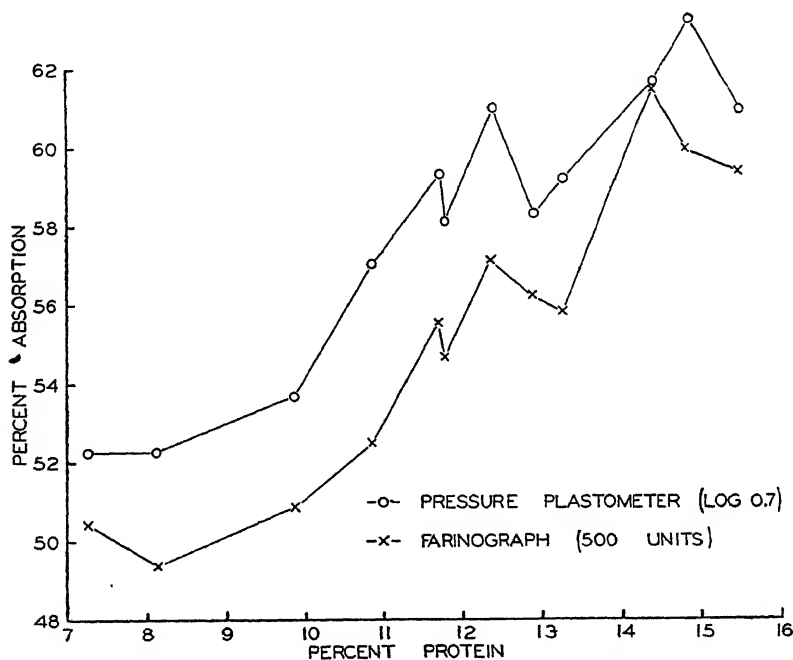
Fig. 2. Relationship of absorption to protein content of various flours as based on measurements on the farinograph and the pressure plastometer.

tions of Stamberg and Merritt (1941). The doughs used in the pressure plastometer were first mixed in the Hobart-Swanson mixer for two minutes and then used at 30° C. The absorptions equivalent to a rate of flow of 5.01 g. per minute (log. 0.7) were determined as described by Stamberg and Bailey (1940).

The graph in Figure 2 shows the relationship between the percentage absorption and the protein content of the flours from the resulting data.

Employing the criteria of a constant rate of flow and a constant plasticity in the farinograph there is obviously an increase in absorption with higher percentages of protein. On the average, this change amounts to about 1.5% absorption per 1% of flour protein.

The results with the farinograph and the plastometer are very much the same and show the same general trend. By selecting other reference standards than 500 F.U., or rate of flow per minute, the curves could be made almost to coincide.

Baking Tests

Baking tests were carried out at various moisture levels for each flour. The formula used included 1% salt, 5% sugar, and 3% yeast. The doughs were mixed for 2 minutes in the Hobart-Swanson mixer. A survey bake was made with the 12 flours using 1½, 2, and 2½ hours' fermentation to find the optimum time, with the results that the three flours of lowest protein content were given 1½ hours of fermentation and all others 2½ hours. The proofing period was 55 minutes at 30°, followed by baking for 25 minutes at 230° C. A mechanical sheeter was used for punching and molding. The low-form baking pans described by Markley (1940) were used.

Each flour was baked at various absorption levels, in one case using a 150 g. portion of dough and in the other case using all of the dough obtained from a unit weight of flour dry matter with the consequent variation in water. The loaves were scored as to volume, texture, grain, and loaf type and expressed in single quality scores, but these values were practically in direct relationship to the loaf volumes which have been used in the data plotted in Figure 3.

It is interesting to note from Figure 3 that when a constant weight of dough was used the larger loaf volumes were reached with increased percentages of absorption, followed by decreased loaf volumes with higher absorption, except in the case of the two soft-wheat flours with 7.26% and 8.12% protein, in which cases the handling property of the dough was the limiting factor. However, when all of the dough was used such maximum loaf volumes followed by decreased values are not apparent. The highest absorptions used were about 64% and 65% on the 15% moisture basis stated in terms of the fully corrected method. The absorption would be much higher if stated on the less accurate dry-matter-corrected or " as is " methods, or on the basis of 13.5% moisture. Thus sufficiently high absorption levels were studied.

The baking data show that when a constant quantity of dough was used the optimum absorptions for the flours were at about 57%–58% on the 15% moisture basis and with the formula and method used.

This indicates that on the accurately calculated and fully corrected absorption value at the 15% moisture level, the optimum absorption is about the same for widely different flours.

Taking the optimum absorption values from the baking data for which a constant quantity of dough was used, a series of farinograph tests was made. These absorption values were all close to 58%. In
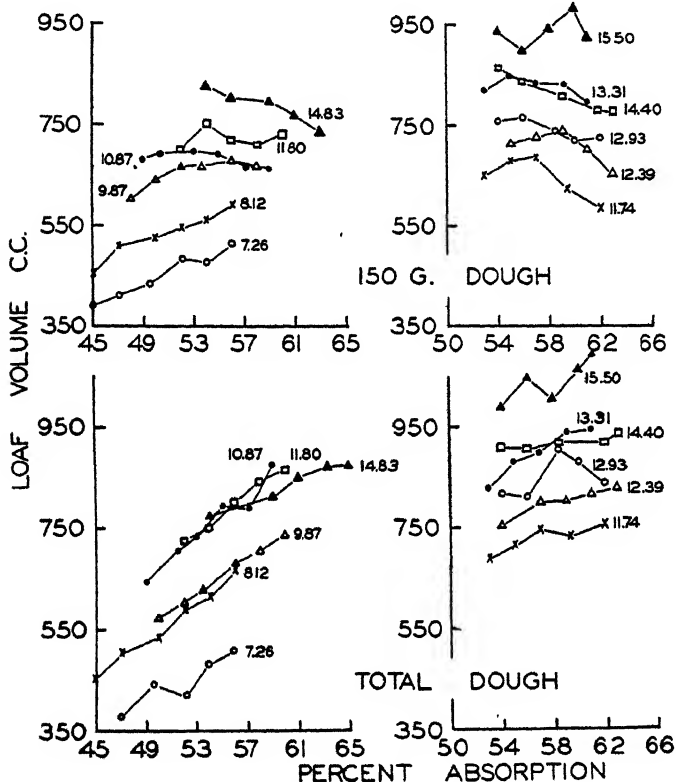
Fig. 3.   Loaf volumes at various absorption levels of 12 flours with different protein contents, the upper graphs with a constant amount of dough, and the lower graphs with all of the dough from 85 g. of flour, dry basis.

Figure 4 the resulting maximum plasticity of the farinograph curve is plotted against the protein content of the flour. It is evident that when optimum absorptions are used on the basis of the baking results the plasticity of the dough increases with higher protein contents of the flours. This is substantially in agreement with the results of Merritt and Bailey (1939), showing that weak flours produce the best baking

results at absorptions giving a low plasticity in the farinograph and stronger flours at an absorption giving correspondingly higher plasticities. Many practical bakers also mix low-protein flours to give slacker doughs, and high-protein flours to give stiffer doughs for best results.

The data in Figure 3 indicate the interesting possibility that for test baking a fully corrected absorption of 58% on the 15% moisture basis
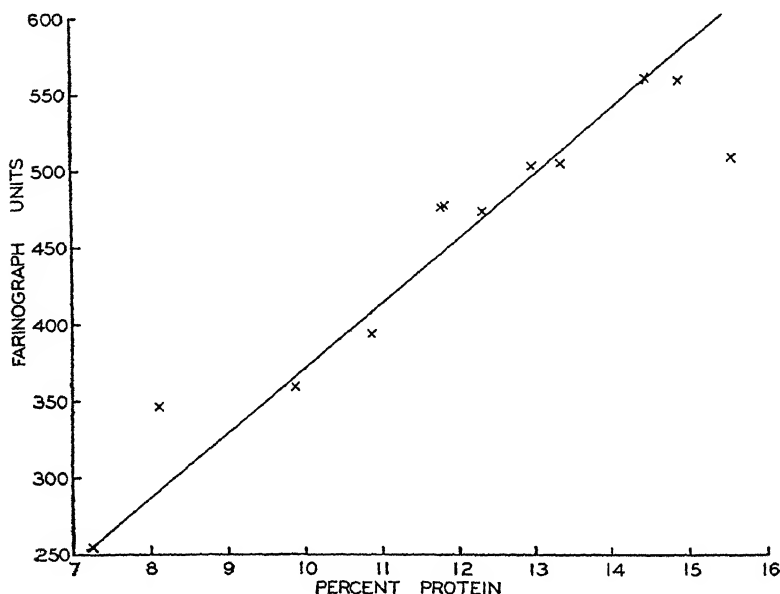
Fig. 4. Relationship between protein content of flour and maximum plasticity, in farinograph units, of doughs mixed at the absorption level found to be optimum by the baking tests for the different flours.

could be used for all flours, and particularly when a constant quantity of dough is used, thus eliminating variations in absorption. It is claimed by many that a better spread in values or differentiation of flour quality is obtained when all of the dough is used in test baking. Such possibilities as are suggested in this paragraph must, of course, be examined with numerous samples of flour.

Summary

Calculation of absorption values of flours by three methods are in general use but only one is entirely correct.

Absorptions of twelve flours, ranging from 7.26% to 15.50% protein content, necessary to have a constant plasticity in the farinograph and

a constant flow in the pressure plastometer, were found to increase about 1.5% for each 1% increase of protein content.

Baking tests were carried out using the flours at various absorption levels, and at each level loaves were baked first with a constant amount of dough of 150 g. and, secondly, from all of the dough resulting from 85 g. of flour, dry basis. With the first method, peaks of optimum absorption were observed as judged from the bread quality, but with the second method no peaks were noticed over the absorption range studied.

Optimum absorptions resulting from the baking tests with a constant amount of dough were found to give doughs of low plasticity in the farinograph for low-protein flours and increasingly higher plasticities for higher-protein flours.

The possibility of using a constant absorption and a constant amount of dough in test baking for flour quality is suggested.

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THE EFFECT OF PROTEIN CONTENT ON THE BAKING BEHAVIOR OF SOME WINTER WHEAT VARIETIES [1]

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Since protein content is the most significant single factor influencing baking performance of sound flours, a consideration of protein content in the correct evaluation of wheat samples is important. Larmour, Working, and Ofelt (1939, 1940) reported upon a series of Kansas-grown varieties of the 1938 crop. Their studies (1939) demonstrated "that within a given season the potential strength of the principal hard red winter varieties is related to protein content in a linear fashion, and is very highly correlated with it." In a second paper (1940) dealing with soft winter wheat varieties these workers stated that a linear relation between loaf volume and protein content did not exist because the loaf volume tended to recede above approximately 10% flour protein in the soft wheat varieties studied.

The study reported at this time was undertaken with two objectives in mind: (*a*) to see if the conclusions reported by Larmour, Working, and Ofelt (1939, 1940) would hold for the 1939 Kansas crop, and (*b*), to observe the effect of the use of a uniform mixing time upon the behavior of a series of flours of varying protein amounts and qualities. An excellent series of wheat samples was made available for this study through the co-operation of A. L. Clapp of the Department of Agronomy.

A review of the literature at this time appears unnecessary because of the comprehensive review given by Larmour (1940). He concluded that the lack of linearity formerly thought to exist between protein content and loaf volume was due to inadequate baking methods. Recently, McCalla (1940), in a study of hard red spring wheats, concluded that the protein content-loaf volume regression coefficient for a given variety is as much a varietal characteristic as is yield or protein content. High-protein Reward wheat (15% and above) made bread of large volume, equal to or better than Marquis, but low-protein Reward was not much, if any, better than Garnet. Garnet required more protein at all levels than Marquis or Red Bobs in order to make a loaf of specified volume.

Anderson, Sallans, and Ayre (1938) used linear regressions with scatter diagrams effectively to show the relationship of nitrogen and sac-

charifying activities in a study of varietal differences in barleys and malts. A similar arrangement proved useful in showing results graphically in the present study (Fig. 3).

Materials and Methods

Samples of the principal varieties of wheat grown in Kansas were produced by farmers in large plots from pure seed supplied by Kansas State College at 59 locations in Kansas. Hard red winter varieties included were Turkey, Tenmarq, Cheyenne, Kanred, Nebred, Early Blackhull, Blackhull, Chiefkan, and Iobred, whereas Kawvale, Michigan Wonder, and Clarkan represented the soft red winter wheats.

Small samples of each variety were harvested and shipped to the Experiment Station for threshing so that acre yields, protein content and other determinations could be made. The samples according to variety were then composited according to their protein contents at about the following protein levels:

Below 10%	14.6 to 16.0%
10.1 to 11.5%	16.1 to 17.5%
11.6 to 13.0%	17.6% and above
13.1 to 14.5%	

These composites were large enough to provide a milling sample in most cases. Five or more flours of each variety were available for baking excepting Nebred and Iobred. It was recognized that this method of making the composites produced samples which were not strictly comparable, as the same locations were not necessarily represented in the various protein levels of the different varieties. Differences in growing environments may account for some of the inexplicable baking results obtained. Samples weighing less than 53 pounds per bushel were discarded as well as all musty or excessively shrivelled samples. The samples were thoroughly mixed and then milled on a Buhler mill. The straight-grade flours were analyzed, stored in tightly covered containers at room temperatures for two weeks and then kept in a cold room at approximately 41°F. until baked.

The flour samples were baked by two methods differing only in the length of mixing time. In one method the doughs were mixed for three minutes at 80 rpm. regardless of the handling properties or stage of development of the doughs. The three-minute period (240 revolutions of mixer) selected as the fixed time was based on a preliminary study involving varieties of widely different mixing times. In the other method the doughs were mixed at 80 rpm. to the optimum consistency as determined by observation. A Swanson-Working dough mixer with a bowl containing two adjacent pins was used.

The baking formula used in both methods was as follows:

Flour	100 g.
Yeast	3 g.
Salt	1 g.
Sugar	6 g.
Malt syrup (120° L.)	0.25 g.
Dry milk solids	6 g.
Shortening (hydrogenated vegetable)	3 g.
Potassium bromate	0.004 g.
Water (distilled)	As needed

Absorption values as determined for the optimum mixing method were also used in the fixed-mixing-time bakes. The standard A.A.C.C. baking test fermentation times and temperatures were employed. Loaves were baked in an electric oven for 24 minutes at 430°F. Fermentation bowls, fermentation cabinet, tall-form baking pans, and the loaf volume measuring apparatus used complied with the requirements of the A.A.C.C. method. A National pup sheeting roll was used for punching, and molding was done on a Thompson Model A laboratory molder. Loaf volumes were measured immediately after the loaves were removed from the oven. The final loaf volumes reported are averages of at least two loaves from two bakes made on different days. Additional replicated bakes were made in cases where the volumes differed more than 20 cc. from each other. The loaves were scored the following day for external and internal characteristics.

Baking absorption values were determined by adding proportionate amounts of the same ingredients used in the baking formula to 20 g. of flour in the bowl of a National micro mixer. Distilled water was added and the mixer operated until the proper consistency as determined by "sight and feel" of dough was attained. The resulting doughs were then fermented for three hours and the final desired baking absorption then estimated from the consistency of the dough. This method is similar to that devised and used by Karl F. Finney[3] of the Federal Hard Winter Wheat Quality Laboratory.

Dough-mixer curves were made for all flours, using a National-Swanson-Working recording micro mixer. All curves were made at the No. 9 setting. Thirty-five g. of flour was used and distilled water was added to equal the absorptions used in baking.

The time-test determinations were made according to the procedure described by Swanson (1937). Test weight, protein, moisture, and ash determinations were made by approved methods. The percentages of protein and of ash are reported on a 15% moisture basis.

Discussion of Results

Analytical data for wheat and flour are given in Table I.   These data show low correlation between protein content and the results obtained by the time test.   There was, however, a tendency for longer time to be associated with higher protein content.   Percentages of flour yield and flour ash varied somewhat, being influenced undoubtedly by milling and by test weight.   Absorption values, while somewhat irregular, showed a definite trend to increase as the amount of protein increased.   Typical soft wheats such as Michigan Wonder or Clarkan had lower absorptions than good hard varieties.   Kawvale absorbed more water

TABLE I

ANALYTICAL DATA

Serial No.	Wheat					Flour			
	Test weight	Flour yield [1]	Time test	Moisture	Protein [1]	Moisture	Protein [1]	Ash [1]	Baking absorption
	lbs.	%	min.	%	%	%	%	%	%
TURKEY									
25095	—	69.4	74	10.6	9.7	14.0	8.8	0.453	56.2
96	58.4	70.8	72	10.8	10.6	13.8	9.5	0.485	55.8
97	57.8	69.3	67	10.6	11.9	13.7	10.5	0.457	57.6
98	57.5	69.7	101	10.6	13.8	13.6	12.7	0.461	59.4
99	57.5	68.5	99	11.0	14.7	14.1	13.9	0.497	63.3
100	56.8	67.9	115	10.2	16.6	13.4	15.7	0.503	63.9
101	57.1	68.3	115	10.4	17.5	13.5	16.7	0.490	65.1
TENMARQ									
89	58.2	66.1	135	10.6	10.8	14.6	9.8	0.416	60.2
88	—	70.2	123	10.5	11.1	14.2	10.7	0.440	59.5
90	58.5	66.9	110	10.6	11.9	13.8	11.2	0.432	58.8
91	59.6	69.2	100	10.8	13.4	13.7	12.3	0.413	59.6
92	57.6	67.9	125	10.4	14.9	13.4	13.7	0.404	61.0
93	57.1	66.5	145	10.2	15.7	13.5	15.3	0.437	63.1
94	56.1	64.4	145	10.4	17.6	14.0	16.6	0.474	67.0
KANRED									
63	57.6	71.1	72	10.4	10.9	14.2	10.2	0.450	60.5
64	57.8	72.2	72	10.6	11.9	13.9	10.8	0.409	59.9
65	57.8	67.9	71	10.5	13.6	14.3	12.5	0.410	62.7
66	57.4	70.2	57	10.5	14.9	13.8	13.9	0.434	63.7
67	59.4	69.2	90	10.8	16.3	14.2	15.5	0.444	66.4
68	58.2	67.7	73	10.2	18.0	13.9	16.4	0.474	66.8

[1] Moisture basis 15%.

TABLE I—*Continued*

Serial No.	Wheat					Flour			
	Test weight	Flour yield [1]	Time test	Moisture	Protein [1]	Moisture	Protein [1]	Ash [1]	Baking absorption
	lbs.	%	*min.*	%	%	%	%	%	%
CHEYENNE									
25049	58.8	69.0	122	10.6	10.6	13.9	10.1	0.430	56.0
50	57.8	72.1	98	11.3	11.9	13.6	10.9	0.417	56.4
51	59.5	69.6	125	10.5	13.3	13.7	12.1	0.441	57.6
52	59.2	68.8	104	10.6	14.9	14.0	13.9	0.453	59.1
53	59.6	67.3	114	10.5	16.3	13.8	15.3	0.465	60.7
54	58.6	70.9	132	10.5	17.3	13.5	16.2	0.505	62.1
NEBRED									
84	—	68.7	121	10.4	13.5	14.1	12.7	0.519	62.3
85	60.1	69.5	125	10.4	14.8	13.8	13.9	0.450	60.7
86	58.6	68.6	154	10.3	16.7	13.4	15.8	0.507	62.0
87	57.6	70.4	176	10.4	17.7	12.9	16.9	0.531	62.0
EARLY BLACKHULL									
35	—	66.5	77	10.3	10.9	13.9	10.4	0.438	56.0
36	58.6	66.4	56	10.6	12.0	14.6	11.5	0.394	58.3
37	60.8	62.7	56	10.7	13.4	14.3	12.6	0.387	58.7
38	60.0	64.0	66	9.9	15.0	14.0	14.6	0.411	59.1
39	58.1	65.0	91	11.1	16.2	13.7	15.6	0.435	59.6
BLACKHULL									
29	59.4	65.7	63	10.4	11.9	14.8	11.2	0.401	58.6
30	59.0	64.7	78	10.3	13.8	13.7	12.3	0.414	56.6
31	58.6	62.0	54	10.3	14.9	14.4	14.4	0.427	61.9
32	59.0	62.8	74	10.4	16.4	14.4	15.8	0.441	61.9
33	58.1	62.2	111	10.2	17.2	14.4	16.7	0.457	63.8
CHIEFKAN									
42	61.0	70.7	60	10.7	11.1	14.0	10.7	0.423	61.1
43	60.6	71.0	58	10.6	11.8	13.8	11.1	0.434	61.7
44	59.4	71.8	61	10.1	13.2	14.0	12.8	0.405	63.1
45	59.6	70.1	61	10.5	15.0	14.6	14.2	0.436	64.2
46	59.3	69.0	70	10.3	16.1	13.6	15.6	0.545	63.3
47	59.9	66.6	65	10.3	16.9	14.2	16.1	0.466	62.5

TABLE I—*Continued*

Serial No.	Wheat					Flour			
	Test weight	Flour yield [1]	Time test	Moisture	Protein [1]	Moisture	Protein [1]	Ash [1]	Baking absorption
	lbs.	%	min.	%	%	%	%	%	%
IOBRED									
25076	60.0	65.0	78	10.6	13.8	13.9	12.7	0.355	58.9
77	—	62.8	—	10.5	15.2	13.5	14.0	0.350	60.2
KAWVALE									
70	58.3	69.0	71	10.5	10.5	13.8	10.2	0.462	58.8
71	56.0	68.2	75	10.7	12.2	13.6	11.2	0.449	61.3
72	56.6	66.6	83	10.7	13.6	13.8	12.3	0.418	62.7
73	56.0	65.8	128	10.7	15.2	13.3	14.0	0.443	62.7
74	55.0	68.1	104	10.6	15.4	13.5	14.5	0.470	65.1
MICHIGAN WONDER									
79	57.0	61.8	42	10.4	10.5	13.3	10.1	0.396	55.9
80	57.6	61.4	54	10.3	11.9	13.2	10.8	0.401	56.7
81	58.2	63.3	59	10.3	13.4	13.0	12.3	0.399	57.3
82	56.8	68.3	70	10.4	14.7	13.6	13.5	0.437	60.4
83	56.8	60.7	76	10.2	15.8	13.0	15.0	0.422	59.3
CLARKAN									
56	59.6	54.2	49	10.4	10.9	14.1	10.0	0.350	57.3
57	60.3	56.7	53	10.2	12.2	13.7	10.4	0.371	56.6
58	59.1	60.5	51	10.3	13.4	13.7	11.9	0.382	56.6
59	59.1	61.3	71	10.4	15.1	13.4	13.6	0.379	57.0
60	58.1	61.5	74	10.3	16.5	13.2	15.4	0.407	57.7

[1] Moisture basis 15%.

than the typical soft wheats.   It is evident that varieties cause definite differences in absorption values.

Figures 1 and 2 show the loaf volumes for the various varieties at different protein levels, excepting Nebred and Iobred, for which less than five levels of protein were available.   Neither of these two varieties proved outstanding and their inclusion would have merely confused the figures.   Loaf volumes for Nebred with the fixed mixing time were, with increasing protein content, 855, 868, 1,072, and 1,112 cc.; with optimum mixing time, 815, 870, 1,033, and 1,145 cc. respectively.   Similarly, with Iobred the results were 898 and 1,013 cc. for the fixed mixing time, and 895 and 975 cc. for the optimum mixes.
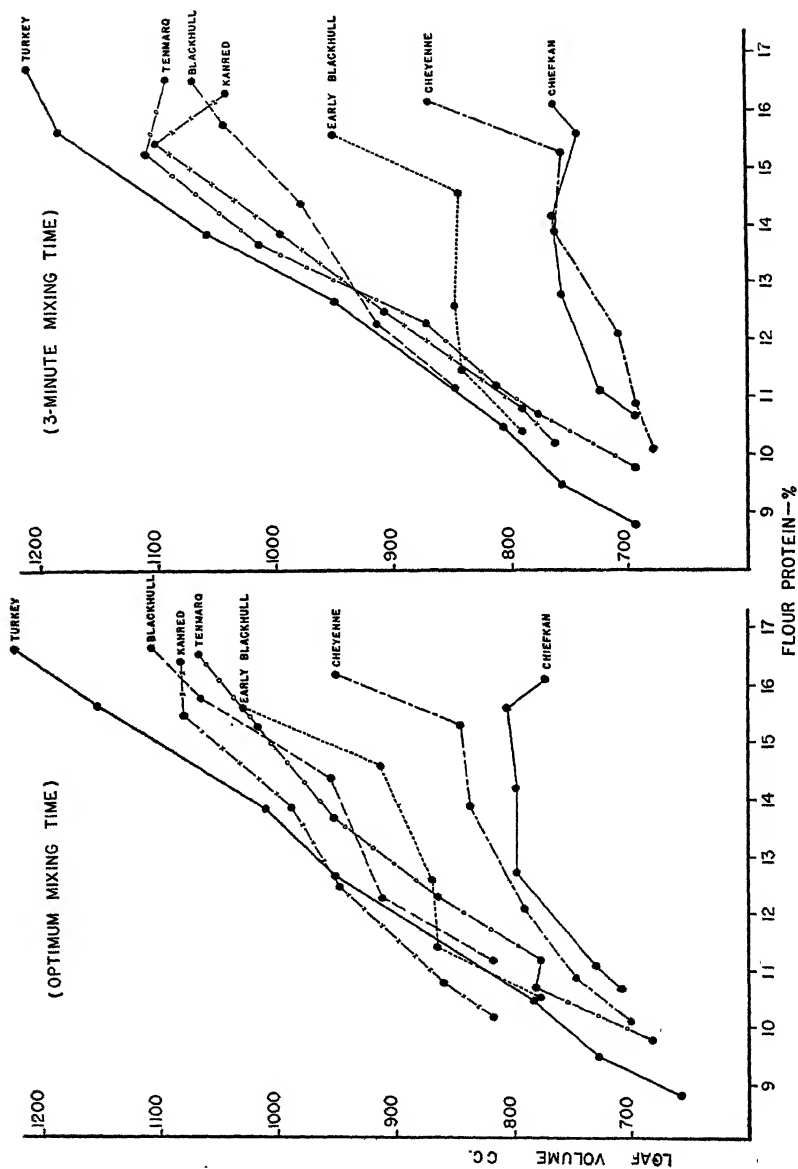
Fig. 1. Loaf volumes for hard red winter wheats at various protein levels (1939).
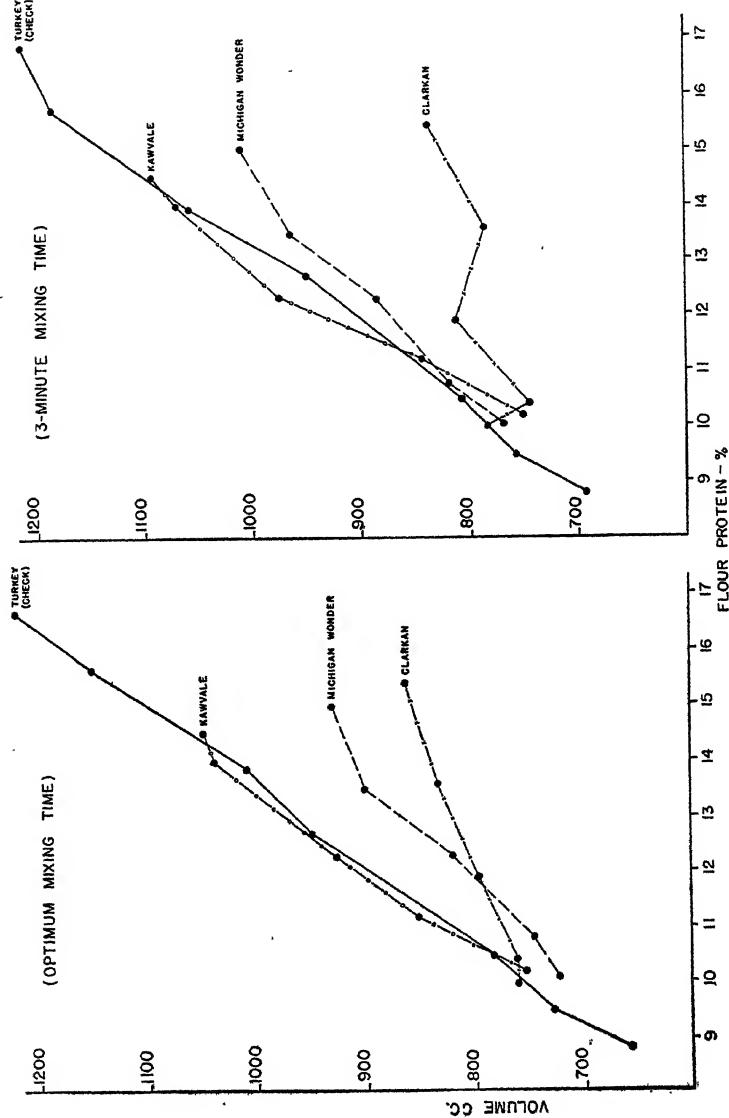
Fig. 2. Loaf volumes for soft winter wheats at various protein levels (1939).

With the fixed three-minute mixing method linearity between loaf volume and protein content is evident in most of the varieties studied (Fig. 3). Turkey, Tenmarq, Cheyenne, Kanred, Blackhull, Kawvale, and Michigan Wonder may be noted in this respect. With Clarkan and Chiefkan, increasing protein content had the least effect upon the loaf

Fig. 3. Protein-loaf volume regression lines from two baking procedures.
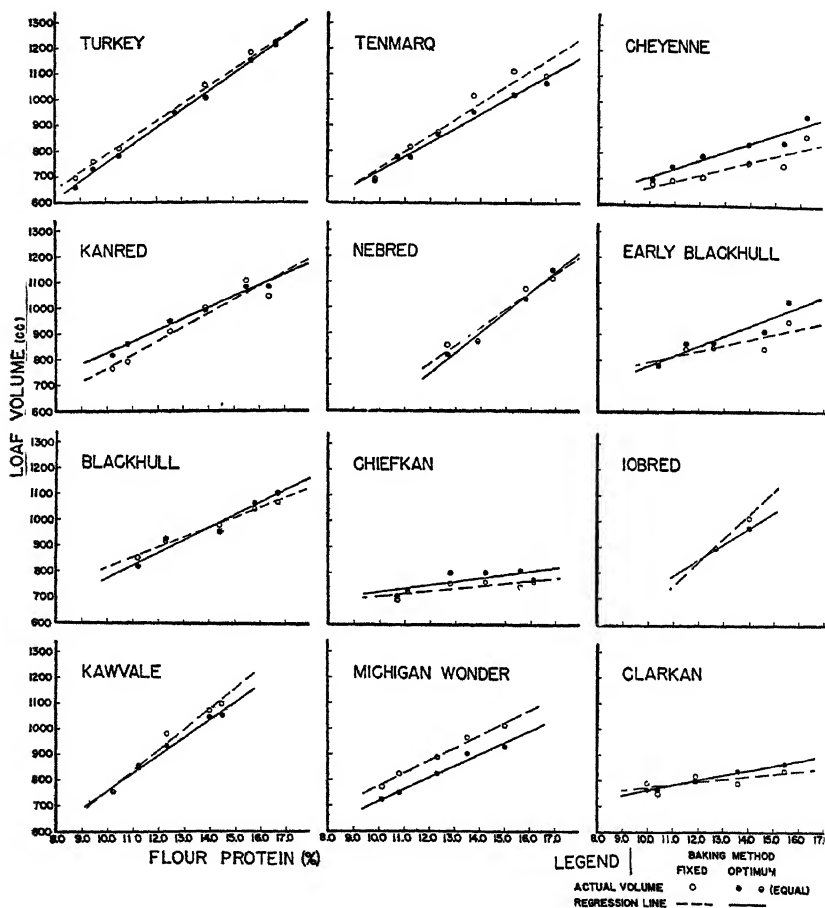
volume. No reason is evident for the peculiar behavior exhibited by Early Blackhull. The figures indicate linearity between loaf volume and protein content in the same varieties when baked with the optimum mixing time as with the fixed mixing time. The loaf volumes of Clarkan and Chiefkan were little affected by the protein content with either mix-

ing time.   Generally speaking, it would appear that the use of the optimum mix tends to improve somewhat the behavior of such varieties as Chiefkan, Early Blackhull, and Cheyenne, particularly at the higher levels of protein.

Cheyenne was the only variety noticeably undermixed with the fixed mixing time (240 revolutions of mixer).   The doughs were dead and underdeveloped.   By mixing to the optimum the handling properties of the doughs of Cheyenne and the resulting bread were improved.   Chiefkan, Blackhull, Early Blackhull, Michigan Wonder, and Clarkan with three minutes of mixing time generally produced doughs that were overmixed, sticky, and difficult to handle.   However, these varieties, even when overmixed, produced bread similar in quality to that from doughs mixed to the optimum.   The optimum mixing time required varied between 1.75 and 5.0 minutes for the entire series of samples and generally the mixing time, within a variety, tended to decrease as the protein content increased.   Examination of the curves (Figs. 4 and 5) shows this same tendency for decreasing mixing requirements with increasing protein content.

A linear relationship between protein content and loaf volume for most varieties included in this study has already been indicated.   However, it is also evident from Figures 1 and 2 that the ranking of the varieties is somewhat different for each baking method at each protein level.   In this respect the baking methods cannot absolutely be used interchangeably in experimental baking for rating varieties as to baking quality.   It is interesting to note that some of these flours when baked by Sandstedt and Ofelt (1940) at Lincoln, Nebraska, with a formula similar (except for type of malt used) to that used in these studies, gave results and rankings different from these same flours when baked at Manhattan, Kansas.   Sandstedt and Ofelt used malted wheat flour as the diastatic supplement.   Methods of handling the doughs also differed somewhat.   It becomes increasingly evident that great care is needed in the choice of test procedures to evaluate varieties so that these varieties will be well adapted to their uses by the consuming public.

Type of Regression Lines

McCalla (1940) has pointed out that the relation between protein content of wheat and loaf volume of bread is just as much a variety characteristic as are yield per acre and protein content.   Data presented in this paper seem to substantiate the above-mentioned relationship in regard to hard red winter varieties.   Regression lines were calculated by a method given by Fisher (1936) to investigate this point.   Figure 3 shows the actual loaf volumes as well as regression lines for each mixing
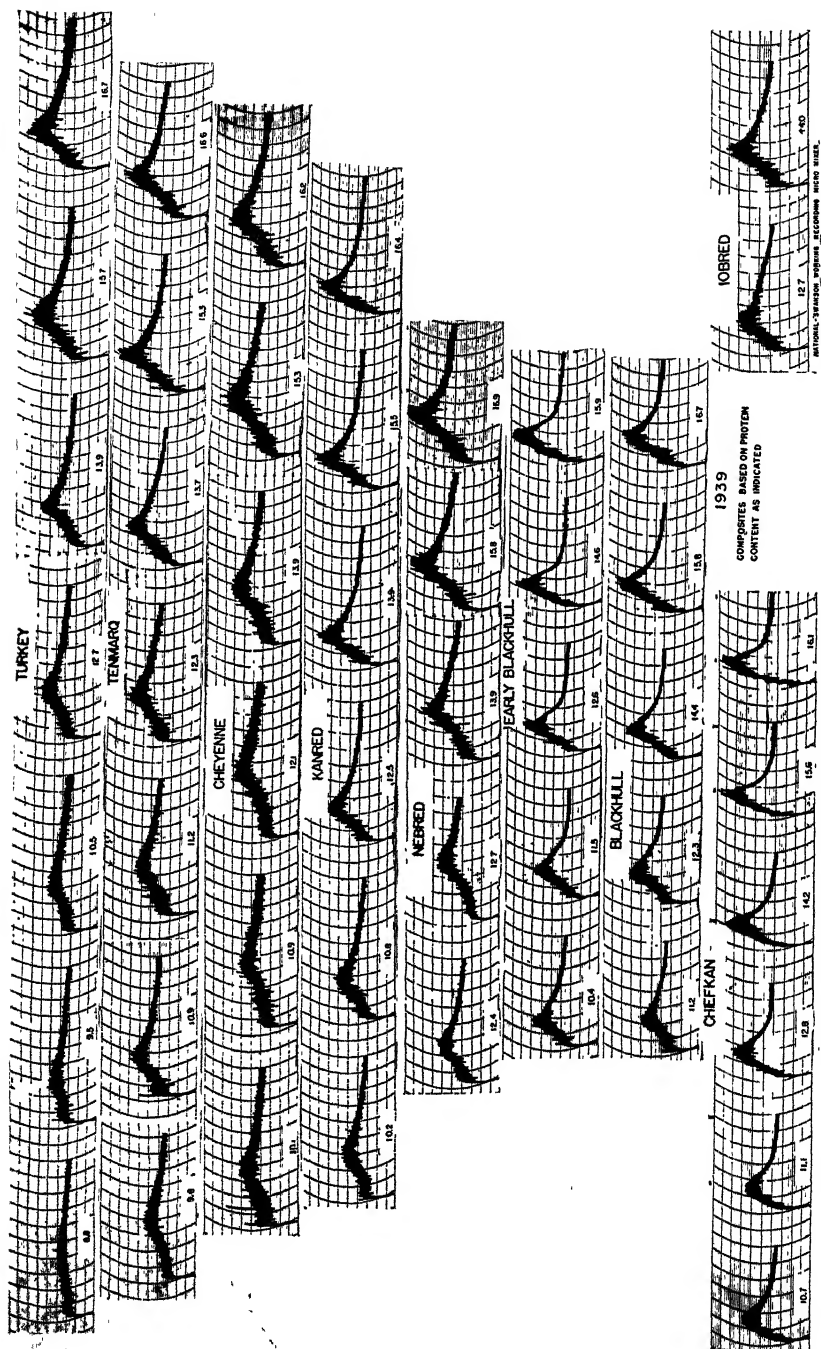
Fig. 4. Micro-mixer curves of hard red winter wheat flours of varying protein levels.

method. The actual data fit the calculated regression lines very well in most instances.

These regression lines show distinctly that the loaf volumes of the different varieties are not affected to the same extent by increases in protein content. These regression lines may be grouped into three general types: (1) lines which demonstrate that loaf volume is relatively poor at lower protein levels and is greatly improved at the higher protein levels, *e.g.* Nebred; (2) lines which demonstrate that loaf volume is relatively good at low protein levels and improves little with increasing protein content, *e.g.* Chiefkan and Clarkan; and (3) lines which demonstrate a uniform and considerable increase in loaf volume in relation to
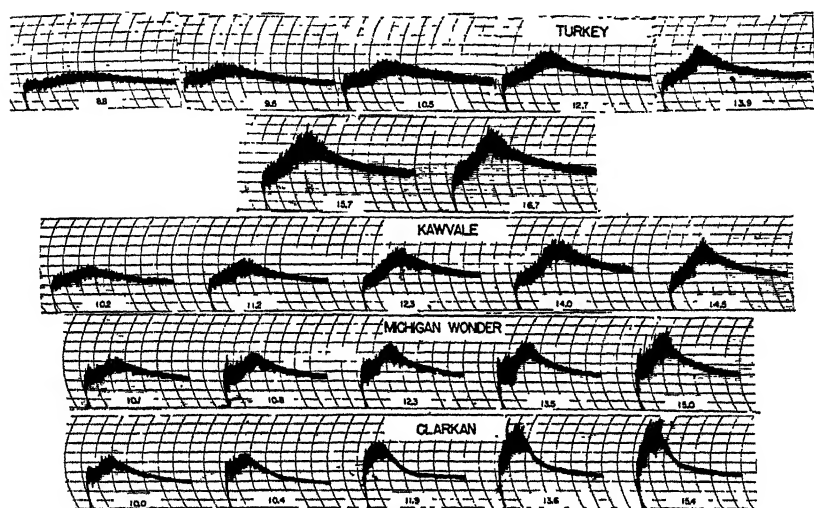
Fig. 5. Micro-mixer curves of Turkey, Kawvale (a semihard variety) and two soft winter wheats, Michigan Wonder and Clarkan.

protein content throughout the entire protein series, *e.g.* Turkey. The baking performances of varieties with regression lines similar to Turkey may be more readily forecast from a protein determination than the performance of varieties possessing either of the other types of regression lines. Further work with hard red winter wheats will be needed to determine definitely that type of regression line is an inherited characteristic in this class of wheat. It is evident that the baking method will influence the type of line to a material extent.

Micro Mixer Curves

Mixing curves for these flours are shown in Figures 4 and 5. These were obtained on the Swanson-Working recording micro mixer made

by the National Manufacturing Company. This machine differs from the original recording micro mixer used by Larmour, Working, and Ofelt (1939) chiefly in that the chart paper is drawn through three times as fast, making each division equal to about one minute of mixing time. The mixing head also rotates at a slightly higher speed. The combined effect of these two changes is that the curves are spread out to approximately twice the length as compared with the curves made with the original model of the recording micro mixer.

No entirely satisfactory method has so far been developed for describing micro-mixer curves. A reliable interpretive method whereby the curves may be expressed numerically is needed. The curves give a pictorial expression of some dough-handling properties of a flour and in this respect the micro mixer provides information about a flour which hitherto has been lacking. Dough-handling properties are factors of great importance to the baker using commercial machinery. They are of less importance in the laboratory testing of samples. Geddes, Aitken, and Fisher (1940) and Geddes (1940) have pointed out that dough-handling properties are not necessarily correlated with the characteristics of the baked loaf. That this is true is exemplified by the curve type and baking behavior of Cheyenne.

Curves indicate approximately, in the time required to reach the peak of the curve, the relative amount of mixing the sample will need in the baking test if an optimum mix is to be employed. In baking the present series of samples a tendency towards shorter time with increasing protein content was noted for the optimum mixing time method. From the curves it may be observed that less time was also required to attain optimum development (peak of curve) in the higher-protein samples.

Examination of the curves shows that the type or pattern changes somewhat with the variation of protein within a variety but the pattern differences between varieties are greater than within a variety. Within a variety the pattern is more or less characteristic although some varieties tend to resemble others of similar dough-handling properties. Early Blackhull and Chiefkan are fairly similar and both differ from Blackhull, which possesses better handling properties. Blackhull curves, however, differ to a considerable extent from either Tenmarq or Turkey. The curve pattern of Kawvale resembles Turkey much more closely than either of the soft winter varieties, Michigan Wonder and Clarkan. In the trade, Kawvale has proved acceptable for family trade flours but is definitely not suitable for high-quality soft-wheat flours used for either cake or soda cracker production.

Examination of the curves inevitably raises the question as to which type is to be considered most desirable. Such a decision is entirely dependent upon the market use of the flour. Among Kansas mills, Turkey

is widely favored and flours with the characteristics of Turkey find ready acceptance in the baking trade. Any variety that does not differ too widely in characteristics from Turkey should therefore also prove satisfactory. Permissible deviation from a standard without causing consumer complaint will depend upon the degree of specialization and standardization in the consuming market. A bakery accustomed to Blackhull type of flour may easily object to Tenmarq flour as being too strong. On the other hand, if Turkey were the accustomed standard flour, then Kanred might be substituted without complaint although Cheyenne might be objectionable because of its excessively long mixing requirement. The difference between Clarkan and Michigan Wonder may be so much that the two varieties cannot be readily interchanged. Many users of soft-wheat flour require a good quality of gluten, even though in small amount, rather than a larger amount of poorer gluten. Additional information is needed as to the desirable curve type for the various specialized purposes for which soft-wheat flours are used.

Summary

Winter wheat of several varieties grown at various places in Kansas was so composited that for each variety a series of samples differing in protein level became available.

Each sample was milled and the flour subjected to two baking tests which differed only in that one method employed optimum mixing time and the other a fixed mixing period of three minutes. The rankings of the varieties from the two baking methods were not identical. The differences were most evident at the lower protein levels. It is considered that these data are not conclusive as to whether an optimum mixing time is essential for the satisfactory evaluation of wheat varieties.

The data indicate the existence of a linear relationship between protein content and loaf volume. This is more obvious in some varieties than in others.

Protein-loaf volume regression lines indicate that these varieties differ in gluten quality. Some varieties are relatively poorer or relatively better in protein quality than others at equal protein levels.

Micro-mixer curves with optimum baking absorptions, indicating dough-handling properties, show that large differences in these properties exist among the varieties studied. These differences are large enough so that some of the varieties probably could not be used interchangeably without causing serious trouble in commercial bakeshops.

Chiefkan and Cheyenne gave the poorest baking results, as indicated by smaller loaf volume, among the hard wheat varieties.

The high baking quality and the curve type of Kawvale show that its quality *as a soft wheat* is poor.   This variety grades red winter on the market.

Clarkan and Michigan Wonder differ materially in baking performance and curve type although both are graded as soft winter varieties.

Acknowledgment

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FOREIGN MATTER IN CORN MEAL

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Cereals contaminated with rodent excreta or by insect infestation are unfit for food. A product such as corn meal may be contaminated because it has been made from filthy corn, or because an infestation has developed in the meal itself. Insects in whole corn will be broken to bits when the corn is ground but the meal is still contaminated with their fragments and their excreta. The same is true of grain containing rodent excreta. Grinding does not remove the excreta. It changes only their shape and makes both insects and excreta less readily detectable by a casual examination.

Some time ago it became evident that corn meal might contain such ground-up filth, and a method was devised to detect the contamination. This procedure is based on a principle employed in other filth-extraction methods issued by the Food and Drug Administration, certain details of which have been specifically modified to insure a clean flotation of insects and rodent hairs with many types of corn meal. In addition, there has been inserted a procedure for the sedimentation of heavy filth. The removal of rodent and insect contamination involves a separation based on the unequal density of the food and filth particles and the affinity of insects and animal hairs to oils.

Many insect excreta are heavier than the cereals, but on the other hand, there are light excreta pellets and some heavier cereal tissues which render a specific gravity separation impractical. When flour is cleared in oil, the pellets, which remain opaque, stand out by contrast. Rodent-excreta-pellet fragments are generally heavier than the comminuted cereals; in a liquid with a specific gravity near 1.49 they will tend to settle out while much of the cereal floats. As the specific gravity is raised, more cereal is floated, but some pellet fragments will also rise and be lost. It is necessary to strike a practical balance between the need to float the plant tissue and the possible loss of excreta. Factors other than density play a part in this separation. The particles must be soaked in the liquid to give them time to become fully permeated. To work the excreta loose from the cereal tissue, the mixtures must be stirred. The density balance is quite delicate and beakers should be covered and otherwise handled to avoid strong convection currents. Rather than attempting to complete the separation with one decantation, it should be repeated as may be necessary. During these decantings, one

should keep the beakers tilted on edge.   In this manner the heavy filth will be in one mass, easily watched and controlled.

Insects, insect fragments, and rodent hairs are often lighter than the cereal and sometimes may be floated out in heavier-than-water liquids while the plant tissue settles out. Usually, however, they are extracted by a different procedure. With the exception of fly larvae, or maggots, insects and insect fragments can be wet with oils mixed into an aqueous mixture of a food and so floated up to the surface with the oil. In practice, because of several factors, this separation is not complete. It is difficult to wet all of the insect material without creating a frothy emulsion of the plant material that will obscure the filth particles in the subsequent microscopic examination. Fragments may become trapped in or attached to a mass of plant material settling out. Droplets of oil often adhere to the sides of the trap flask and may hold insects there, thus keeping them from rising. To reduce some of these effects, the oil or gasoline is worked thoroughly into the water-cereal mixture, but with no " whipping " and as little inclusion of air as possible. Intermittent agitation is provided while the separation is taking place. Some products cannot be extracted in water because too much of the food rises with the " light filth." To reduce some of these effects, the oil or gasoline is worked thoroughly into the water-cereal mixture, but with no " whipping " and as little inclusion of air as possible. Intermittent agitation is provided while the separation is taking place. Some products cannot be extracted in water because too much of the food rises with the " light filth." To reduce floury emulsions, the extractions can be made in saturated salt solution. Sometimes caprylic alcohol or 95% ethyl alcohol can be used to break an emulsion. In general, when much bran or chaff is present, it will float up with the oil when water or saturated salt is used and it is advisable to do the extracting in a water-ethanol solution. (For some cereals a water-isopropyl alcohol solution may be used.) The alcohol not only soaks into bran but it is also less dense, so that less plant tissue floats. Material trapped off in one Wildman trap [1] may be transferred to another trap and rewashed to remove some of the plant material. While this is necessary in many instances, any nonessential operations may lose filth material and are to be avoided.

Filter papers should be so treated that the microscopic examination is as simple as possible. To accomplish this it is advisable to (1) clear any extraneous plant material that may be present, (2) prepare excreta for examination, and (3) use coarse filters that have been marked off into areas to facilitate examination. Flour and bran may be cleared with mineral oil or chloral hydrate, thus rendering the insect fragments and

[1] First described by B. J. Howard in *Food Industries*, July, 1935.

rodent hairs more readily visible. If mineral oil is used, the material on the filter must be air-dried before the oil is added. Mineral oil is well suited to the microscopic determination of insects, insect fragments, and rodent hairs, but in it rodent excreta become so hard it is difficult to open the excreta to find the rodent hairs. Hence the sediment from a chloroform extraction should be cleared and softened in 75% alcohol. If an excessive amount of starchy material is present, the paper may be completely cleared by gelatinizing it with chloral hydrate. The chloral leaves the pellet fragments soft but is extremely noxious to work with and should be washed out of the filter after the clearing is complete and before the microscopic examination is made.

The filter paper should be ruled in fine parallel lines 6 to 7 mm apart. Filter paper may be purchased with water-, alcohol-, and oil-proof lines printed on it or the lines may be applied conveniently by means of a rubber stamp and pad. Waterproof India ink makes a permanent nonspreading line. If the filter paper is not ruled, it is necessary to place a wire grid over the paper to mark it off into smaller areas.

In testing corn meal, care should be taken to secure representative samples.[2] They should be fumigated at once with chloroform or carbon disulfide vapor or held under refrigeration to prevent any living insects from working in the sample.

Procedure

Separation of rodent excreta by sedimentation: Weigh out a 50-g portion of the well-mixed sample into a 250-ml hooked-lip beaker. Add chloroform to within ½ inch of the top, mix thoroughly and allow to settle for at least 30 minutes. Several times during this period stir the layer that rises to the top. Decant the chloroform and the floating corn tissue into a 7-cm Buchner funnel attached to a suction flask and containing a smooth-surface filter paper, being careful not to disturb the heavy residue in the bottom of the beaker. Before decanting, take care that the floating layer has not become so compact that this operation is difficult. Keeping the beaker tilted on edge, add chloroform, allow to stand for several minutes, and again decant. Add carbon tetrachloride in an amount equal to the chloroform and corn tissue left in the beaker, allow to settle again, and decant as before. Repeat this process with a mixture of equal parts of chloroform and carbon tetrachloride until very little corn tissue is left in the beaker. Avoid decanting any rodent excreta fragments that may be present. At this point the bulk of the corn meal will be in the Buchner funnel and the heavy residue, including the rodent excreta fragments, if any, will remain in the beaker.

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[2] W. G. Helsel and Kenton L. Harris, Method for the Recovery of Filth from Corn Meal (Tentative), U. S. Food and Drug Administration. Mimeographed. Revised December 12, 1939.

The residue in the beaker is now washed onto a 7-cm filter paper with a stream of chloroform or carbon tetrachloride. Carefully transfer the filter paper to a petri dish. Immediately before the microscopic examination is made, add sufficient 70% alcohol to wet the filter paper and material on it thoroughly while not causing the corn and filth particles to flow. This alcohol will soften the rodent excreta pellet fragments and clear the corn tissue.

Separation of rodent hairs and insects by flotation: Draw air through the Buchner funnel until the chloroform-carbon-tetrachloride mixture is evaporated. Carefully transfer the contents of the funnel onto a large sheet of clean, smooth paper. If the material is clumped, dry off the remaining chloroform in the air or in an oven at 60°–65°C. Transfer this material to a one-liter Wildman trap flask. Add 100 ml of 60% ethyl alcohol or 53% isopropyl alcohol (by volume) and mix thoroughly. (This alcohol is used with the usual type of whole corn meal, which may or may not have been sifted and/or bolted. In case degerminated or cream meal is being tested, or meal that has had the bran and chaff removed by bolting and/or aspiration, the same procedure is followed except that distilled water or a saturated salt solution is used instead of the 60% alcohol, and only 25 ml of gasoline is required.) Wash down the sides of the flask with a stream of 60% alcohol from a wash bottle and allow the material to soak for 30 minutes. Add 35–40 ml of gasoline, mix thoroughly, and allow to stand for 5 minutes. While stirring, add more 60% alcohol until the floating gasoline layer carrying the filth and some corn tissue can be trapped off in the neck of the flask. Allow to stand for 30 minutes, stirring 4 to 6 times during this period in order to release filth fragments trapped in the bottom of the flask, and also to permit some of the corn tissue to settle out of the neck of the flask. (If a large amount of emulsion has formed in the neck, it may be broken with a very fine stream of 95% alcohol from a wash bottle.) After a few minutes, raise the stopper into the neck of the flask and by gentle agitation release any corn tissue that has begun to settle out. The gasoline layer must be allowed to separate completely again before it is trapped off. Carefully spin the stopper to remove corn meal from its top and trap off the filth by drawing the stopper tightly into the neck of the flask. The gasoline and about ⅓ to ½ inch of the alcohol layer should be trapped off above the stopper and filtered through a rapid-acting filter paper in a Buchner funnel.

Rinse the contents of the neck of the flask carefully into the funnel—first with alcohol and then with water. Wash down the sides of the Buchner funnel with a stream of alcohol and continue suction until the paper is dry. Hold the funnel tilted against the edge of a petri dish

containing a few drops of mineral oil and carefully remove the filter paper to the dish. This oil serves to clear the corn tissue, making the filth fragments more readily visible. Add 20 ml of gasoline to the material in the flask and stir vigorously to draw the gasoline down into the mixture. Add sufficient 60% alcohol to bring the gasoline layer well into the neck of the flask. (If distilled water or salt solution was used in the first extraction it should be substituted here.) Allow to stand for 10 minutes before again trapping off into a Buchner funnel and transferring to a petri dish.

Identification and recording of filth: After clearing, the material on the filter papers is ready for examination with a Greenough type binocular dissecting microscope using 20 to 30 magnifications. A white background and reflected light should be employed. The following may be recovered from the residue left after the chloroform-carbon-tetrachloride decanting: rodent excreta fragments (identified by the presence of pieces of rodent hairs), a few insect fragments (especially those embedded in rodent or insect feces), a small number of hairs, and nondescript filth fragments. From the gasoline extraction may be recovered rodent hair fragments, a few hairs with pieces of excreta attached, insects, and insect fragments. If there is any doubt about the identification of any of the fragments, they should be removed to a slide and examined under the compound microscope.

Count and record the findings as follows:

> Rodent-excreta-pellet fragments
> Detached rodent hair fragments
> Adult insects
> Pupae
> Detached adult insect heads
> Insect larvae
> Cast skins and capsules
> Detached insect larvae heads
> Miscellaneous insect fragments
> Nondescript filth fragments

It is essential that the analyst be able to recognize the extracted filth, and proficiency in this direction can be gained only through an examination of authentic samples. Various food-infesting insects should be collected and studied. To learn what they look like in comminuted products, individual insects should be carefully dissected *in detail* and the various parts studied at 20 to 30 magnifications so that their fragments can be recognized. One of the references given later may be helpful as a guide in this work. Analyses should not be attempted until several

species have been worked over.   Insects develop in four stages, the egg, larva, pupa, and adult bearing little resemblance to each other.   Each stage should be separately studied.   It is suggested that the confused flour beetle, saw-toothed grain beetle, granary weevils, Angoumois grain moth or Indian meal moth, meal worms, and mites be the main objects of study.   Little or no attention need be paid to the internal anatomy.

Similarly, rodent and other types of hairs should be examined.   Hairs embedded in excreta and in fragments of excreta should be compared with material of known origin.   Insect excreta may be readily obtained from some of the more common storage insects which may be reared conveniently in the laboratory.   Types from various kinds of insects should be examined.

The resolving power of the compound microscope is superior to that of the Greenough type binocular and during the beginning work it should be used frequently at 100 to 200 magnifications to study details of structure.   As progress is made, it may be used with less frequency.

After whole and dissected insects and rodent filth have been studied, the same types may be studied after being ground in a mortar.   Authentic material must be used.   A study may then be made of tissues of corn and wheat.   (See the books by Winton referred to later.)   The kernels should be examined with the naked eye and then studied as carefully as were the contaminants.   At this point, differences between the plant and insect tissues should be noted.

After this work has been completed, the methods for extracting filth from foods should be studied.   By practicing with grossly contaminated samples, it is possible to develop the manual technique and, at the same time, extract much material for subsequent microscopic observation.   This comparative work must be continually checked back to authentic material.

Obviously the technician should be thoroughly familiar with the proper use of the microscope in the identification of filth.   One of the best books available for this purpose is *The Microscope,* by S. H. Gage (Comstock Publishing Co., Ithaca, N. Y.), 1932.   For identifying plant structures the *Textbook of General Botany,* by R. M. Holman and W. W. Robbins, 1939, is suitable, but for the details of seed structure, books such as the following may be consulted: *The Microscopy of Vegetable Foods,* by A. L. Winton, 1916, and *The Structure and Composition of Foods,* Vol. I, by Winton and Winton, 1932.   The latter three books are published by John Wiley and Sons, New York.

Often it will be desirable to use some references in studying the contaminants.   For guidance in the insect study, *An Introduction to Entomology,* by J. H. Comstock (Comstock Publishing Co., Ithaca, N. Y.), 1920, may be used.   *Principles of Insect Morphology,* by R. E. Snod-

grass (McGraw-Hill Book Co., N. Y.), 1935, is recommended only as a detailed, highly technical reference book. There are several excellent volumes that can be used as a general guide to the classification of insects, but the U. S. Department of Agriculture Farmers' Bulletin 1260, *Stored Grain Pests,* 1938, probably will be sufficiently complete for general use. U. S. Department of Agriculture Miscellaneous Publication 318, *4–H Club Insect Manual,* 1940, may be helpful, and Miscellaneous Publication 258, *Annotated List of the Insects and Mites Associated with Stored Grain and Cereal Products,* 1937, gives a more complete list of references. Both these and the following references on hairs are amply illustrated.

The microscopic characteristics of animal hairs are discussed in several publications. *A Study of Hairs and Wool,* by John Glaister, Misr Press, Cairo, Egypt, 1931, contains an extensive discussion of the structural characteristics of the hairs of many mammals including rodents, cats, dogs, and human beings. The *Journal of Wildlife Management,* Vol. II, pp. 239–250, 1938, contains an article on mole and shrew hairs and a concise review of the microscopical features of hairs and hair types. For a description of some common hairs and the methods of studying them, there is an article in the February 21, 1920, *Scientific American,* page 200, by L. A. Hausman. Similar material concerning fur hairs appears in an article by the same author in the January, 1920, *Scientific Monthly,* page 70, while more complete data on a variety of mammals appear in his excellent *American Naturalist* article, " Structural Characteristics of the Hair of Mammals," page 496, 1920. Some further details of hair structure, perhaps of interest to the research worker, appear in the *American Naturalist* for November–December, 1924, page 544.

THE DETERMINATION OF NICOTINIC ACID IN BREAD AND OTHER CEREAL PRODUCTS

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Since the discovery by Elvehjem and coworkers of Wisconsin in 1937 that nicotinic acid is a vitamin and specific for the prevention and cure of pellagra, there has been a demand for a rapid, accurate method for the determination of nicotinic acid applicable to bread and cereal products.

Bandier and Hald (1939) found that metol (*p*-methyl amino phenol sulfate) plus nicotinic acid and cyanogen bromide in aqueous solution

yields a clear yellow color which is perfectly stable for a period sufficient to be read, and of an intensity directly proportional to the amount of nicotinic acid. We tried this method and found it applicable to the determination of nicotinic acid in yeast if extreme caution is observed, but not applicable to cereal products like flour and bread. In determining nicotinic acid in products to which the method is applicable we found that even minute residual quantities of acetone used in the process vitiated the results and that it was very difficult to remove the last traces of this solvent. The color technique employed was not satisfactory because of the high blank values and turbidity, which introduced a source of error.

In the determination of nicotinic acid in yeast by the Bandier and Hald method reasonably accurate results were obtained, but in many instances the values were erratic. When 0.66 μg of nicotinic acid per gram was added to yeast, 0.69 μg per gram of yeast was found. Since yeast also contains nicotinic acid, the difference represents about the normal amount contained in the yeast before the addition. When this method is applied to flour, bread, and other cereal products a semisolid mass results which will not produce an extract suitable for subsequent analytical treatment.

It occurred to us that this semisolid mass could be liquefied by the application of diastase, since the treatment given was sufficient to prepare the starchy material for diastasis. This proved true and we found that any suitable diastatic reagent could be used for the purpose. We employed takadiastase and found it quite suitable for the liquefaction. Since takadiastase is also used in the Hennessy method for the determination of thiamin, we thought its use as the diastatic reagent in our process would avoid the introduction of another reagent.

The subsequent treatment of the sample by the Bandier and Hald procedure, where acetone is employed, produced turbidity and high blank values that proved unsatisfactory for cereal products after treatment with diastase.

The method of Arnold, Schreffler, and Lipsius (1940) for the determination of nicotinic acid is not applicable to flour, bread, and cereal products, but their color production technique for the treatment of our liquefied extract from diastase was found applicable for the reading of the solutions. These authors used p-aminoacetophenone and cyanogen bromide to produce a stable color that gives a relatively low extinction coefficient in the blank, free from turbidity and other interfering sources of error.

Experimental

Preparation of extract: A sample of the bread to be assayed for nicotinic acid is sliced and preferably dried in a hot-air oven (80°–90°C) overnight.[1] A moisture determination is made so that the bread can be calculated to a definite moisture content. The dried bread is reduced to fine particle size with mortar and pestle or is ground. Twelve grams are placed in a centrifuge bottle and suspended in 75 ml of distilled water. The sample is then autoclaved for 10 to 15 minutes at 15 pounds pressure. After cooling to 50°–60°C, 0.3 g of takadiastase is added and held at 50°–60°C for one hour, centrifuged, and the supernatant fluid decanted into a 100-ml graduate. The residue is shaken up with 30 ml of distilled water, centrifuged and added to the first extract. The total liquid of about 80-ml volume is transferred to a 125-ml Erlenmeyer flask, 5 ml of concentrated HCl added, and the extract heated for 30 minutes on a boiling water bath. After cooling, 20% NaOH is added to bring the pH to approximately 4. After standing for 5 minutes the liquid is transferred to a centrifuge bottle and centrifuged at high speed for 10 minutes. The clear solution is then brought to a pH of 6 with 20% NaOH and transferred to a 100-ml graduate. The volume of 85–90 ml is accurately noted, as this volume measurement is used in computing the nicotinic acid concentration of the entire sample.

Color production: If the original moist bread contains around 10 mg of nicotinic acid per pound, 5-ml portions are measured into each of the four 15-ml amber glass graduated cylinders; if only about 5 mg of nicotinic acid is contained per pound, use 8- to 10-ml aliquot portions. Twenty micrograms of nicotinic acid are added to one of the flasks and 40 to another. All four cylinders are then heated in a hot water bath (80°C) for 10 minutes and then 2 ml of cyanogen bromide added (saturated bromine water just decolorized with 10% KCN in the cold) to three of the cylinders, including the two cylinders with added nicotinic acid. After heating an additional 4 minutes at 80° C, the cylinders are cooled rapidly to room temperature, and after 4 minutes 0.2 ml *p*-amino-acetophenone is added to each cylinder (10 g dissolved in 28 ml of 10% HCl diluted to 100 ml). The graduates are shaken, placed in the dark for 15 minutes, and then 0.4 ml of 10% HCl is added from a micro-burette, after which they are allowed to stand 15 minutes more in the dark. The volume of each graduate is made up to 13 ml with distilled water. The mixtures are then transferred to a 25-ml centrifuge separatory funnel (Pfaltz & Bauer, Inc.) containing 15 ml of ethyl acetate. The funnels are shaken for exactly 5 minutes and the water layer is then

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[1] The bread sample does not have to be dried for the determination, but only to give more accurate data for the calculation and to give a higher concentration of nicotinic acid per unit weight of bread.

drawn off and discarded. The ethyl acetate is clarified with 2 g of anhydrous Na_2SO_4 whereupon it is ready for colorimetric reading. A Pfaltz & Bauer fluorophotometer is used with a combination blue and yellow filter to give a wave length of about $420\,\mu$. The light intensity is adjusted to read zero extinction with ethyl acetate with switch in transmission position. The extinction value of the sample is then determined. The blank reading is subtracted from the other values obtained. The corrected values are then plotted on graph paper and the value of the nicotinic acid in the original sample thus obtained (Fig. 1).
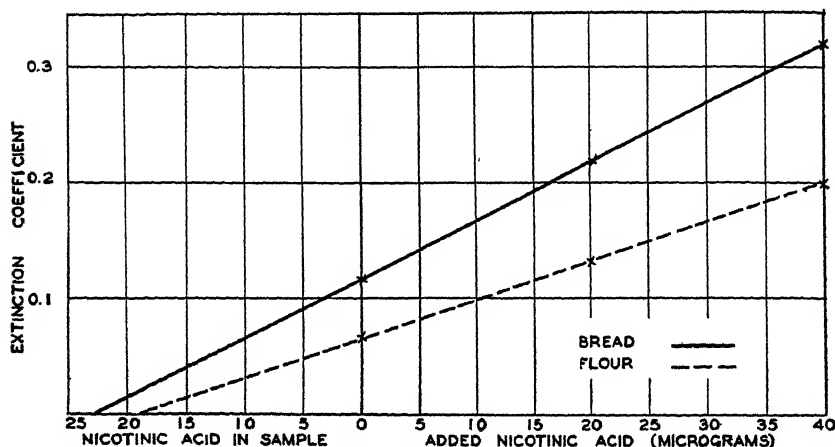
Fig. 1. Data used in the nicotinic acid method.

Bread data:

Weight dried bread used	12.0 g
Volume of extract	85.0 ml
Volume of extract used	5 0 ml
Bread moisture	34.0 %
Nicotinic acid content	9.75 mg/lb

Flour data:

Weight flour used	10.0 g
Volume of extract	100.0 ml
Volume of extract used	10 0 ml
Nicotinic acid superimposed	10 μg
Nicotinic acid content	3.85 mg/lb

Comparative data (basis 1 lb bread):

	Nicotinic acid in Yeast	Bread	Flour (diff.)
(1)	7.55 mg	9.75 mg	2.20 mg
(2)	6.05 mg	8.60 mg	2.55 mg

Nicotinic acid in flour by assay ... . 3.85 mg/lb
Nicotinic acid in bread (calculated) .... 2.42 mg/lb

Galvanometer readings:

Bread		(Corrected)	Flour		(Corrected)
B 0.034			B 0.035		
0 . . . . 0.149	0 115		0 . . .. 0.101	0 066	
20 . ... 0.254	0.22		20 . ... 0 166	0.131	
40 .... 0.352	0.318		40 .... 0.234	0.199	

Recovery of added nicotinic acid in bread: Samples of bread used in this experiment were divided into two groups. Group A had 5 mg of added nicotinic acid per pound of moist bread. Group B had 10 mg of

added nicotinic acid per pound of moist bread. The following results were obtained:

Sample No.	Group A mg nicotinic acid per lb moist bread
1	5.53
2	5.40
3	5.36
4	5.48
5	5.42

Sample No.	Group B mg nicotinic acid per lb moist bread
1	10.85
2	10.90
3	10.76
4	10.83
5	10.79

Determination of nicotinic acid in flour: The determination of nicotinic acid in flour is best carried out by adding a portion, 0.2 g, of takadiastase to the flour sample and heating to 65° to 70°C with stirring; holding at this temperature for 15 minutes, then bringing to a boil and pressure cooking to avoid clumping. The sample is then cooled to 50°–60°C, 0.3 g more takadiastase added, and the liquefaction completed. The procedure from here on is the same as for bread, except 10-ml portions of the extract are used for color development with 10 μg of nicotinic acid superimposed in each portion in addition to the samples containing the added 20 and 40 μg of nicotinic acid. This modification brings the readings up into the desired range.

The method given in this paper appears to be simpler in many details than that proposed by Melnick, Oser, and Siegel at the spring meeting of the American Chemical Society (1941). The color production technique of Arnold, Schreffler, and Lipsius (1940) was used because of the relatively low extinction values obtained in the blanks. Bandier-Hald's color technique was tried but rejected because of the high blank values which introduced a source of error.

Reagents and Apparatus

Reagents:

1. Cyanogen bromide: saturated bromine water just decolorized with 10% potassium cyanide in the cold.
2. *p*-aminoacetophenone: 10 g dissolved in 28 ml of 10% HCl and made up to 100 ml.
3. Ethyl acetate, reagent grade.
4. Sodium sulfate, anhydrous, reagent grade.

Apparatus:

1. Centrifuge bottles: 200–230 ml.
2. Amber glass graduates: 15 ml (Otto R. Greiner Co., Newark, N. J.).
3. Centrifuge separatory funnels: 25 ml (Pfaltz and Bauer, Inc., New York).
4. Fluorophotometer: (Pfaltz & Bauer, Inc., New York).

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THIAMIN CONTENT OF TYPICAL SOFT WHEAT MILL STREAMS

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Within the last few years the determination of thiamin (vitamin B_1) in cereals has presented one of the outstanding problems to the cereal chemist. The oxidation of thiamin to thiochrome and the subsequent quantitative measurement of this fluorescent compound has been utilized by a number of workers with varying degrees of success. Jansen (1936) first devised a procedure for thiamin by this method using quinine sulfate as a standard of fluorescence. Karrer and Kubli (1937) used a pure solution of thiamin oxidized in parallel with the unknown as a standard. Westenbrink and Goudsmit (1937) first removed thiamin from interfering substances by adsorption on franconite and used the method for analysis of urine. Various other modifications have been introduced by Pyke (1937) and Hennessy and Cerecedo (1939). Wang and Harris (1939) encountered thiamin losses in the absorption and elution with zeolite. They removed soluble interfering substances by extracting the thiamin solution with isobutyl alcohol. Booth (1940) used a modified Wang and Harris method on a number of English wheats. Schultz, Atkin, and Frey (1939) have reported the thiamin content of wheat, various grades of flour, bran, shorts, and germ, measured by the yeast fermentation test.

Experimental

Believing that a definite contribution could be made to the knowledge of thiamin in cereals by a determination of the thiamin content of various mill streams, we obtained from a mill employing a five-break, seven-reduction system, representative samples of the flours produced together with typical samples of red dog, shorts, and bran. All samples were milled from the same sample of commercial Ohio soft wheat.

After a preliminary survey of methods the following procedure was adopted from the method of Wang and Harris. Two- to five-gram samples were weighed out into 125-ml Erlenmeyer flasks, and 50 ml of 2% acetic acid was then added and mixed with the sample. The mixture was placed in a boiling water bath for 15 minutes, allowed to cool, and then 5 ml of $1N$ sodium hydroxide was added and mixed well. This was followed by 5 ml of a solution of takadiastase containing 4 g of takadiastase per 100 ml. The mixture was incubated at 42°–45°C for 90–100 minutes and then centrifuged until the supernatant fluid was clear.

The clear solution was decanted off into a cylinder containing 20 ml of isobutyl alcohol and shaken vigorously for one minute. The alcoholic and aqueous layers were allowed to separate by gravity or by centrifuging. The solutions were then poured carefully into a separatory funnel, the lower aqueous layer drawn off, and the alcoholic layer discarded.

Three aliquots of the aqueous extract were taken for oxidation. The three aliquots were placed in 125-ml separatory funnels. One-tenth to 0.25 ml of 1% potassium ferricyanide (depending upon the thiamin concentration) was added and mixed, followed by 3 ml of 15% sodium hydroxide and immediately 15 ml of isobutyl alcohol, with mixing after each addition. The samples were then vigorously shaken for 1½ minutes and allowed to stand until the layers separated. The third aliquot was used as a blank. It was treated like the other two except that the potassium ferricyanide was omitted and the alcohol was added before the sodium hydroxide.

After the two layers had separated, the aqueous layer was drawn off and discarded. The isobutyl layer containing the thiochrome was run into a test tube and 1 ml of 95% ethyl alcohol added to clarify it. The clear solution was compared in a Pfaltz and Bauer fluorometer, with a quinine sulfate solution used as a standard. This standard was checked daily against a solution containing one microgram of thiamin which was oxidized by the above procedure just previous to comparison. This method was found to check closely with the method of Hennessy and Cerecedo (1939) using zeolite. The thiamin content was calculated by

the equation:

$$\mu g/g = Dx/Ds \times 60/A \times 1/S$$

Where: $Dx =$ galvanometer deflection of unknown

$Ds =$ deflection of one microgram standard of thiamin

$A =$ ml in aliquot

$S =$ weight of sample in grams

$60 =$ volume sample extract

Amounts of thiamin in the various mill streams are given in Table I. Accuracy of the method was determined by a recovery test made by adding a known amount of thiamin to a sample previously analyzed and repeating the determination on the reinforced sample. Since all the

TABLE I

DISTRIBUTION OF THIAMIN IN THE VARIOUS MILL STREAMS

Laboratory number	Stream	Thiamin (μg per g)		Ash content, 13.5% moisture basis
		Found [1]	Corrected (times 1.11)	
				%
1179	1st break flour	0.8	0.9	0.404
1180	2nd break flour	1.0	1.1	0.406
1181	3rd break flour	0.8	0.9	0.474
1182	4th break flour	0.7	0.8	0.480
1183	5th break flour	1.3	1.4	0.590
1184	1st mids flour	0.9	1.0	0.338
1185	2nd mids flour	0.6	0.7	0.350
1186	4th mids flour	0.8	0.9	0.376
1187	5th mids flour	1.3	1.4	0.424
1188	6th mids flour	2.0	2.2	0.478
1189	7th mids flour	2.4	2.6	0.648
1190	1st tailings flour	1.1	1.2	0.535
1191	2nd tailings flour	1.8	2.0	0.560
1192	Sizings flour	0.7	0.8	0.326
1193	Bran and shorts dust reel	2.4	2.6	0.740
1194	1, 2, 3 breaks, hex. reel cuts	1.2	1.3	0.840
1195	Low-grade flour	3.6	3.9	0.684
1196	3rd mids patent flour	0.7	0.8	0.328
1197	3rd mids clear flour	0.9	1.0	0.358
1198	Red dog	9.2	10.1	—
1199	Shorts	9.3	10.2	—
1200	Bran	5.2	5.7	—

[1] At least two analyses on different days, checking within 10% of each other, were used in obtaining the average results.

percentage recoveries, shown in Table II, on all samples investigated were from 88% to 91% it was believed safe to multiply each result by the factor 1.11 to get the true thiamin value.

Some work was done on the necessity for hydrolyzing possible phosphate complexes of thiamin with takadiastase. If little or no phosphory-

TABLE II

RECOVERY OF ADDED THIAMIN

Labora-tory number	Stream	Micrograms of thiamin			Recovery	
		In sample	Added	Found	Micro-grams	%
1179	1st break flour	4.0	20	21.6	17.6	88
1183	5th break flour	6.5	20	24.2	17.7	89
1190	1st tails flour	5.5	20	23.7	18.2	91
1192	Sizings flour	3.5	20	21.1	17.6	88
1195	Low-grade flour	10.8	20	29.0	18.2	91
1197	3rd mids clear flour	4.5	20	22.5	18.0	90
1198	Red dog	18.4	20	36.2	17.8	89
1199	Shorts	18.6	20	36.2	17.6	88

lated thiamin occurs in cereal products this step could be omitted with much saving of time. The procedure used was the same as previously outlined except that the addition of $1N$ sodium hydroxide and takadiastase and the incubation at 45° were omitted. Results are given in Table III.

TABLE III

EFFECT OF TREATMENT WITH TAKADIASTASE

Laboratory number	Stream	Thiamin (μg per g)	
		With takadiastase (uncorrected)	Without takadiastase (uncorrected)
1180	2nd break flour	1.0	1.0
1181	3rd break flour	0.8	1.0
1182	4th break flour	0.7	0.7
1184	1st mids flour	0.9	0.8
1185	2nd mids flour	0.6	0.6
1190	1st tails flour	1.1	1.0
1191	2nd tails flour	1.8	1.5
1192	Sizings flour	0.7	0.6
1195	Low-grade flour	3.6	3.66
1196	3rd mids patent flour	0.7	0.7
1198	Red dog	9.2	9.0
1199	Shorts	9.3	5.8

Apparently the use of takadiastase is unnecessary, at least in flour, although more work should be done on this phase.

Pepsin digestion was investigated with bran, but without increasing the thiamin value, although the blank was higher in the case where pepsin was used, as follows (bran 3-gram sample, 60-ml extract, 5-ml aliquot): *With pepsin:* Reading thiochrome 45, blank 18, net 27. *Without pepsin:* Reading thiochrome 39, blank 13, net 26.

Conclusions

The thiamin content of mill streams from the same sample of Ohio wheat varied widely. In a general way there seems to be a direct relationship between ash content and thiamin content. This is in agreement with the work of Hoffman, Schweitzer, and Dalby (1940), who found a definite relationship between thiamin content and ash content in clear flours.

The method of Hennessy can be shortened for wheat products with no loss of accuracy by the substitution of a preliminary extraction of the unknown solution with isobutyl alcohol for the adsorption on zeolite. It is doubtful that the use of takadiastase is necessary for the analysis of thiamin in wheat flour.

Summary

A number of flours of different mill streams with the bran, shorts, and red dog milled from the same sample of wheat were analyzed for thiamin by a modified thiochrome method. The thiamin content of the flours varied between 0.7–3.9 μg per gram, following roughly their respective ash contents.

It is suggested that takadiastase may be unnecessary in the analytical procedure. Red dog flour contained 10.1 μg per gram, while shorts, which probably contained much of the germ as well as fine bran, contained 10.2 μg per gram and the bran contained 5.7 μg per gram.

Since bran contained about the same concentration of thiamin as whole wheat, it can be left out of bread flour without appreciable sacrifice of thiamin. The shorts and red dog fractions, however, are considerably higher in thiamin than the original wheat; hence their removal causes large losses in the vitamin. The low-grade flours have slightly lower thiamin values than the whole wheat from which they originated.

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THE THIAMIN AND RIBOFLAVIN CONTENTS OF
WHEAT AND CORN [1]

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The chemical methods for the determination of riboflavin and thiamin
which have recently been developed make possible a more comprehensive
study of the occurrence of these vitamins in wheat and corn than would
be feasible with the tedious and costly biological methods.   Such a study
is timely as a result of the current interest in vitamin enrichment of cereal
products to whole-grain levels.   In order to carry out intelligently the
fortification of cereal products, it is necessary to know the variations in
the quantities of the vitamins in the whole grain, and the factors influenc-
ing these variations.   The authors have investigated some of these fac-
tors, using the procedure described in a previous paper (1941) for the
combined determination of thiamin and riboflavin.

Because of limitations inherent in biological methods, many of the
values found in the literature for the thiamin and riboflavin content of
wheat and corn were necessarily based upon assays of small numbers of
samples.   Consequently these data do not indicate the range of vitamin
values that may occur.

Chemical analyses of wheat and corn for thiamin have been reported
recently, but as far as the authors are aware, no such study has been
reported for the riboflavin content of these grains.   Schultz, Atkin, and
Frey (1939, 1941), using the yeast fermentation procedure, reported

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[1] Presented at the American Chemical Society Meeting at St. Louis on April 9, 1941.

thiamin values for wheat samples varying from 4.2 to 7.3 μg per gram, with an average value of 5.6.   Hoffman, Schweitzer, and Dalby (1940), using the same procedure, analyzed 46 samples of whole wheat flour derived from wheat of different varieties and from various localities. They found the average thiamin content to be 6.85 μg per gram.   Booth (1940), employing the thiochrome method, analyzed 78 varieties of wheat, common to the English market.   He has reported values ranging from 1.62 to 9.99 μg of thiamin per gram with an average value of 3.75. A sample of durum wheat had the highest thiamin content, and English spring wheats were generally richer in thiamin than the winter varieties. As Booth's method did not entirely eliminate interfering substances, his figures may be expected to be slightly high.

Riboflavin and Thiamin Contents of Hard and Soft Wheat

Fifteen varieties of hard wheat and 16 varieties of soft wheat were analyzed for thiamin and riboflavin.[2]   The values obtained are shown in Tables I and II.

TABLE I

Riboflavin and Thiamin Content of Varieties of Hard Wheat

Variety	Thiamin	Riboflavin
	μg/g	μg/g
Amber Durum	5.80	1.20
Blackhull	5.84	1.09
Ceres	4.35	1.06
Cheyenne	4.88	1.09
Chiefkan	4.57	1.14
Early Baart	6.90	1.31
Kanred	5.20	1.41
Marquis	4.35	1.31
Montana Marquis	4.98	0.96
Nebraska No. 60	4.00	1.91
Nebred	4.98	1.27
Ridit	3.65	0.94
Tenmarq	5.30	1.03
Turkey	5.72	1.16
Turkey Red	6.05	0.89

The thiamin content of the hard wheat varieties shown in Table I range from 3.65 to 6.90 μg per gram, with an average value of 5.03. The values given in Table II for the soft wheats vary from 2.43 to 4.77 μg of thiamin per gram with an average of 3.52.   The results shown in Tables I and II are in confirmation of Booth's (1940) conclusion that hard wheats have a somewhat higher thiamin content than the soft-

TABLE II

RIBOFLAVIN AND THIAMIN CONTENT OF VARIETIES OF SOFT WHEAT

Variety	Thiamin	Riboflavin
	μg/g	μg/g
Albit	3.91	0.89
Currell	3.25	1.48
Dicklow	3.63	1.24
Federation	3.12	0.81
Fulhio	3.39	0.85
Fultz	2.65	1.02
Gladden	3.65	1.24
Kawvale	3.39	1.06
Leap	3.69	1.30
Purdue No. 1	2.43	1.02
Purplestraw	3.69	1.30
Rex	4.77	1.10
Rudy	3.51	0.82
Thorne	3.67	1.10
Triplet	4.00	1.10
Trumbull	3.71	0.85

kernel types. The Early Baart variety was found to have the highest thiamin content (6.90 μg per gram) among the hard wheat samples; and the Triplet variety was highest among the soft wheats (4.00 μg per gram). The thiamin values given in Tables I and II are in general agreement with those reported for the same varieties by Schultz, Atkin, and Frey (1941).

The hard wheats showed a range of 0.89 to 1.91 μg of riboflavin per gram, with an average value of 1.17. Among the soft wheats the range was 0.81 to 1.48 μg of riboflavin per gram with an average value of 1.07. No significant difference was found in the riboflavin content of hard and soft wheat. As shown in Tables I and II, the riboflavin content of wheat is considerably lower than that of thiamin. This is in confirmation of the result of a single bioassay reported by Morgan and Hunt (1935). However, the values reported in the present paper do not bear out the conclusion reached by Munsell and De Vaney (1933) that the riboflavin content of wheat varies directly with the thiamin content.

Riboflavin and Thiamin Values of Wheat Grown in Different Localities

Wheat samples obtained from the various wheat-producing areas of the United States were analyzed for thiamin and riboflavin. The values reported in Table III are for wheats of unknown variety but are representative of the localities from which they were obtained.

Although for certain areas only a few samples were examined, the general conclusion may be drawn that the locality from which the wheat is obtained has a direct bearing on its thiamin and riboflavin content.

This may in part be due to the kernel type (hard or soft) of the wheat grown in any particular area.

TABLE III

RIBOFLAVIN AND THIAMIN CONTENT OF WHEAT GROWN IN VARIOUS LOCALITIES

State	Number samples studied	Average thiamin	Average riboflavin
		$\mu g/g$	$\mu g/g$
Colorado	1	5.84	1.02
Idaho	1	3.91	0.89
Indiana	14	4.12	1.03
Kansas	7	5.01	1.14
Michigan	11	4.31	1.00
Montana	3	4.52	1.08
Nebraska	10	4.47	1.20
Ohio	3	3.65	1.10
Oklahoma	6	5.32	1.33
Oregon	2	4.66	0.95
Texas	9	3.91	1.42
Virginia	3	3.35	1.34
Washington	6	4.51	1.08

Correlation between Protein, Thiamin, and Riboflavin Content of Wheat

Table IV gives the thiamin and riboflavin content for wheats of different protein content. These values indicate a direct relationship between the protein content of wheat and its thiamin content. The wheat

TABLE IV

CORRELATION BETWEEN PROTEIN, THIAMIN, AND RIBOFLAVIN CONTENT OF WHEAT

Number samples studied	Range of protein content	Average thiamin content	Average riboflavin content
	$\%$	$\mu g/g$	$\mu g/g$
5	7–9	3.93	1.02
14	10–12	4.05	1.24
15	13–15	4.78	1.10
15	16–19	5.03	1.24

samples having the highest protein content also contained the largest amounts of thiamin. No such direct relationship was found with riboflavin.

Influence of Environmental Conditions on Riboflavin and Thiamin Content of Wheat

Through the kindness of Mr. Karl F. Finney of the Hard Winter Wheat Quality Control Laboratory at Manhattan, Kansas, who supplied

the samples, a study was made of the riboflavin and thiamin content of varieties of wheat grown from the same seed but under different environmental conditions. The samples were grown by agricultural experiment stations and agencies cooperating in the winter wheat program. For a detailed description of the varieties and the conditions under which they were grown, the reader is referred to the bulletin, *Comparison of Winter Wheat Varieties Grown in Cooperative Plot and Nursery Experiments in the Hard Winter Wheat Region in 1939*, published in January, 1940, by the Bureau of Plant Industry of the U. S. Department of Agriculture.

TABLE V

THIAMIN AND RIBOFLAVIN VALUES OF WHEAT AS INFLUENCED BY VARIETY AND LOCATION

Locality	Kharkof		Blackhull		Tenmarq		Kawvale × Tenmarq		Oro × Tenmarq		Chiefkan	
	Thia-min	Ribo-flavin	Thia-min	Ribo-flavin	Thia-min	Ribo-flavin	Thia-min	Ribo-flavin	Thia-min	Ribo-flavin	Thia-min	Ribo-flavin
	μg/g		μg/g		μg/g		μg/g		μg/g		μg/g	
Akron, Colorado	—	—	—	—	5.83	1.02	—	—	—	—	—	—
Alliance, Nebraska	4.13	0.95	—	—	4.55	1.19	3.91	0.81	4.35	1.22	4.13	1.22
Chillicothe, Texas	—	—	4.24	1.49	4.13	1.14	3.81	1.31	4.67	1.10	2.97	1.19
Denton, Texas	—	—	3.39	1.99	3.60	1.36	3.08	2.03	3.71	1.19	—	—
Goodwell, Oklahoma	4.75	1.22	5.10	1.19	5.83	1.06	5.83	1.90	5.93	1.69	4.45	1.19
North Platte, Nebraska	—	—	—	—	4.99	1.39	—	—	4.24	1.02	—	—

The results in Table V indicate that environmental conditions during the period of growth affect both the riboflavin and thiamin content of wheat, although Harris (1934), Leong (1939), and Scheunert and Schieblich (1936), using biological methods of assay, have reported that different soil fertilizer treatments were without effect on the thiamin content. It would seem desirable to carry out further studies (utilizing the more rapid chemical procedures) concerning the influence of climate, soil, etc., on the thiamin and riboflavin content of wheat.

Riboflavin and Thiamin Contents of White and Yellow Corn

Determinations of thiamin and riboflavin were made on samples of white and yellow corn obtained from the principal corn-producing areas of the United States. The results are shown in Table VI.

White corn was found to have a slightly higher thiamin content than yellow corn, a difference which was also observed by Akroyd and Roscoe (1929), although Schultz, Atkin, and Frey (1941) found no significant difference. No important difference was found in the riboflavin contents

of the two types. The average thiamin content of all the samples analyzed is somewhat lower than the value of 5.34 μg per gram reported by Schultz, Atkin, and Frey (1941).

TABLE VI

RIBOFLAVIN AND THIAMIN CONTENT OF WHITE AND YELLOW CORN

Kernel type	Number samples	Thiamin		Riboflavin	
		Range	Average	Range	Average
		$\mu g/g$	$\mu g/g$	$\mu g/g$	$\mu g/g$
White	12	2.54–7.40	4.40	0.92–2.29	1.32
Yellow	16	2.33–5.93	3.80	0.84–2.02	1.30

Riboflavin and Thiamin Contents of Various Wheat and Corn Products

Various wheat and corn mill products were analyzed for riboflavin and thiamin with the results given in Table VII. These values indicate that wheat germ is richer than corn germ in thiamin. However, the thiamin contents of the bran portions of these cereals were approximately the same. The riboflavin values of wheat and corn germ were considerably lower than the corresponding thiamin values.

TABLE VII

RIBOFLAVIN AND THIAMIN CONTENT OF VARIOUS WHEAT AND CORN PRODUCTS

Material	Number samples	Thiamin range	Riboflavin range
		$\mu g/g$	$\mu g/g$
Wheat germ	3	30–45	4–5
Corn germ	2	19–33	2–5
Wheat bran	3	5–6	3–4
Corn bran	1	4–5	1–2
Corn oil cake	1	9–10	6–7
Corn grits	5	0.5–1.0	0.3–0.5
White corn meal	3	1–2	0.7–0.8
Yellow corn meal	3	1–2	0.5–1.0

Summary

A study has been made of the thiamin and riboflavin content of wheat and corn, with the following results:

The hard-kernel types of wheat have been found to contain a higher thiamin content than the soft types but, with respect to riboflavin, there was little difference between the two.

In both wheat and corn there was a higher concentration of thiamin than of riboflavin.

The thiamin content of wheat appeared to be dependent on variety, protein content, and the environmental conditions under which it was grown. These factors appeared to be less important in the case of the riboflavin.

The thiamin value of white corn was found to be slightly higher than that of yellow corn, but little difference was found between the two kinds in respect to riboflavin.

Wheat germ had a higher thiamin content than corn germ, but the riboflavin value was approximately the same.

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THE DISTRIBUTION OF VITAMIN E IN PRODUCTS OF CEREAL MILLING [1]

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(Read at the Annual Meeting, May 1941)

Vitamin E is of particular interest to the cereal chemist since some of the richer sources are the oils contained in the embryo of cereal grains. Of these wheat germ oil is the richest known source. Although our knowledge of this vitamin dates back less than twenty years, an extensive literature on the subject has been ably reviewed by Mattill (1939), Bacharach and Drummond (1939), and by Merck & Co. (1940).

The existence of a dietary factor essential to normal reproduction in rats was discovered independently by Evans and Bishop (1922), Mattill (1922), and Sure (1924). Wheat germ oil was found to be a potent source of this substance by Evans and Burr (1925), who designated it as the "anti-sterility vitamin fat soluble E." Its occurrence in other vegetable oils such as rice germ, cottonseed, and lettuce was later established, but animal products such as butter and eggs were found to contain only traces. The vitamin was isolated by Evans, Emerson, and Emerson (1936) from the nonsaponifiable fraction of wheat germ oil, in the form of two alcohols which were designated alpha- and beta-tocopherol respectively from "tokos" (childbirth) "pheros" (to bear) and the ending "ol" indicating an alcohol. Alpha-tocopherol was found to possess the formula $C_{29}H_{50}O_2$. Later these same workers isolated from cottonseed oil a third substance possessing vitamin E activity which they named gamma-tocopherol (Emerson, Emerson, and Evans, 1936).

Alpha-tocopherol was synthesized in 1938 by Karrer et al. (1938), Bergel et al. (1938), and Smith et al. (1938). It differs from the natural compound only in being optically inactive. The three tocopherols are closely related chemically, the beta and gamma forms being isomeric and differing from the alpha only in containing one less methyl group in the aromatic nucleus. No standard unitage such as exists for the other vitamins has as yet been satisfactorily worked out for E, and the potency of such preparations is therefore expressed in terms of the amount required to restore normal reproductive efficiency in E-deficient rats. The biological potencies of alpha-, beta-, and gamma-tocopherols are not identical and, while conflicting statements are to be found in the literature, the generally accepted activities are 1 to 3 mg for the alpha, about 5 mg for the beta, and 7 mg for the gamma form.

Isolation of the naturally occurring tocopherols is accomplished by first preparing the nonsaponifiable matter, removing the bulk of the sterols, and further concentrating by partition between solvents, chromatographic adsorption, or high vacuum distillation. Final isolation and purification are carried out by forming derivatives such as the allophanates, half-succinates, or higher fatty acid esters.

The tocopherols are pale yellow, viscous oils which can be oxidized to tocoquinones of greatly lessened biological activity; solutions in fatty oils, however, are relatively stable to atmospheric oxidation. They possess marked antioxidant activity which has been found to be in inverse relation to biological potency.

Their physiological properties are primarily related to the reproductive functions. In the female rat, lack of vitamin E results in failure of the embryo to develop and subsequent resorption. In the male rat, continued deprivation results in testicular degeneration and complete and permanent sterility. Studies with larger animals and humans have shown very conflicting results and their requirements for this vitamin are still an open question.

In this study, our problem was concerned primarily with estimation of the low concentrations of tocopherol present in oils extracted from products of cereal milling. The animal assay procedure developed by Evans and Burr (1927) and by Palmer (1937) is inapplicable to the determination of the vitamin in such low concentrations. This difficulty may be overcome by feeding a concentrated preparation such as a nonsaponifiable but two inherent objections to the biological method still remain: first, only estimates of *relative* potency can be secured and no inference can be drawn regarding the kind of tocopherol present, and second, extensive preliminary assays are necessary in order to set the proper bioassay level.

These difficulties could be obviated if a satisfactory chemical test were available and, within recent years, several methods for the analytical determination of tocopherol have been proposed. These include the spectrographic procedure used by Drummond and co-workers, but applicable only to relatively pure material; oxidation to the tocoquinone either by gold chloride in a potentiometric titration as described by Karrer *et al.* (1938a); with ferric chloride in the original colorimetric iron-dipyridyl method of Emmerie and Engel (1938), or as modified by Parker and McFarlane (1940); and oxidation to the red-orthoquinone with nitric acid as developed by Furter and Meyer (1939).

Extensive investigation in our laboratory indicated that none of these chemical methods was applicable to the problem because of the presence of large amounts of interfering substances. In the case of the oxidation procedures of Karrer and Emmerie and Engel, the interfering materials

are reducing in nature and cause high results.   Efforts to free the sample of these nontocopherol reducing compounds have not been successful. In the Furter and Meyer method, chromogenic substances are present which yield yellow and brown pigments upon oxidation with nitric acid and thus mask the red color of the orthoquinone.   This latter method was selected as a basis for working out a suitable analytical procedure.

The method finally developed is based upon chromatographic fractionation of the nitric acid oxidation products upon activated alumina and is effective in separating the red orthoquinone oxidation product of tocopherol from the interfering pigments.   This method will be described in detail elsewhere.   It has been checked by extensive comparisons with the bioassay procedure and in the instance of preparations derived from wheat, which are known to contain alpha-tocopherol substantially, excellent agreement between the two methods has been found.   It should be pointed out that no chemical methods yet developed are capable of distinguishing between alpha-, beta-, and gamma-tocopherols, and since the biological activities of these compounds differ widely it is necessary to resort to animal assay coupled with the results of chemical analysis to ascertain if a single tocopherol or a mixture is present.

In conducting such combined chemical and biological tests it is necessary to work with the nonsaponifiable matter rather than the oil itself, since only in this manner is it possible to secure an adequate concentration of the vitamin.   In addition, the presence of large amounts of glycerides interferes with adsorption in the chemical procedure.   Since the tocopherols are quite sensitive to oxidation in the presence of alkali and soaps, it is necessary to carry out these initial steps of saponification and extraction of the nonsaponifiable in such a manner as to permit of quantitative recovery of the vitamin in an unaltered condition.   That this is accomplished by the technique employed is illustrated by the bioassay data in Table I.

TABLE I

COMPARISON OF BIOASSAYS OF WHEAT GERM OIL AND THE NONSAPONIFIABLE
FRACTION DERIVED THEREFROM

| Sample | Level fed | Biological activity | |
		Total litter	Implant
		%	%
Wheat germ oil	500 mg	100	76
Nonsaponifiable = to 500 mg of oil		100	86

In applying the method to the examination of mill products, large samples were extracted in modified glass Soxhlet extractors capable of dealing with three to five kilograms at a charge.   Sufficient quantities of oil were thus extracted to enable both chemical and biological determina-

tions to be carried out upon the nonsaponifiables which were further concentrated by removal of a large part of the sterols present by crystallization from methanol.

The analytical results obtained with a series of hard wheat mill streams and products of milling are detailed in Table II and a comparison with bioassay values in Table III.

TABLE II

MILL YIELD, OIL CONTENT, AND TOCOPHEROL CONTENT AND DISTRIBUTION IN
HARD-WHEAT MILLED PRODUCTS

Sample	Mill yield	Oil	Tocopherol in 100 g		
			In oil	Mill product	Distribution
	%	%	%	mg	% of total
Patent flour	60.3	0.83	0.003	0.03	2.0
First clear flour	9.4	1.78	0.082	1.46	17.4
Second clear flour	4.1	4.16	0.069	2.87	14.8'
Red dog	2.7	5.83	0.099	5.77	20.0
Shorts	9.3	4.41	0.072	3.18	37.6
Bran	14.0	2.97	0.012	0.30	6.3
Germ	0.1	8.90	0.178	15.84	1.9
Whole wheat	—	1.54	0.059	0.91	—

TABLE III

TOCOPHEROL CONTENT AND BIOASSAY DATA UPON NONSAPONIFIABLE FRACTIONS
(Hard-wheat milled products)

Source	Tocopherol in sterol-reduced nonsaponifiable	Level fed	Bioassay	
			Tocopherol in sample fed	Total litter
	%	mg	mg	%
Whole wheat oil	2.34	42.7	1.0	83
Patent flour oil	0.15	212.0	0.3	0
First clear flour oil	4.18	24.0	1.0	57
Second clear flour oil	4.80	20.6	1.0	100
Red dog oil	6.16	16.3	1.0	100
Shorts oil	2.80	35.9	1.0	86
Wheat bran oil	0.29	346.0	1.0	67
Wheat germ oil	12.10	7.3	0.9	100

In considering the results of this study, it must be emphasized that the milled products examined were obtained from a single large mill and may not be entirely representative. In this study, we were as much concerned with establishing the analytical procedure upon a sound basis as with securing data upon the tocopherol content of the various fractions. It is pertinent, therefore, to first consider the comparative data presented in Table III. In these animal assays the amount of non-

saponifiable fed was adjusted so as to contain 1 mg of tocopherol based upon the chemical data, since by the bioassay technique employed in this laboratory, a dosage of 1 mg of alpha-tocopherol results in a total litter efficiency of about 85%. On this basis the majority of the results are in good agreement and, with the possible exception of the first clear flour, lie well within the error of the normal animal assay for vitamin E. These results confirm the relative precision of the analytical method, particularly when it is realized that all the bioassay values fell within the critical region upon the first test, since *no* preliminary " level setting " assays were made. They also indicate that the tocopherol of whole wheat is substantially alpha.

Turning now to the data presented in Table II, it will be noted that the major portion of the tocopherol is contained in the red dog, shorts, and clear flours, the patent flour being practically free and the bran quite low. Together, these latter fractions, while constituting 75% of the mill yield, account for only 8%, at most, of the total tocopherol, whereas the clear flours, shorts, and red dog, which represent 25% of the mill yield, contain 90% of the total, and the germ, with only 0.1% yield, accounts for 2%.

Since the embryo represents approximately 2.5% of the total weight of the kernel, it is obvious that much of the tocopherol found in certain of the milled products is attributable to the presence of pulverized germ. Using the value of 0.91 mg per 100 g as the tocopherol content of whole wheat, calculation indicates that approximately 55% of the total tocopherol is derived from the embryo, leaving 45% unaccounted for. Since the patent flour and bran are very low (8% of the total) it is apparent that the remaining 37% must originate in the tissues from which the clear flours, red dog, and shorts are derived and we thus secure the picture that vitamin E is concentrated in two regions of the wheat kernel, one being the embryo and the other the layers of endosperm closely adjacent to the bran. It must be emphasized again, however, that these results refer only to the products of one mill and the conclusions regarding distribution within the kernel require confirmation by more extended investigation upon a range of samples. It is possible, however, to state fairly definitely that not all the vitam'n E of wheat is contained in the embryo, and also that this " nongerm " tocopherol is substantially alpha.

A study similar to the one described above has been conducted with the commercial milled products of durum wheat, the results being detailed in Tables IV and V.

The bioassay data in Table V also confirm the precision of the analytical results, although the agreement between the two sets of data is not quite as good as was the case with the hard-wheat fractions. The values

TABLE IV

MILL YIELDS, OIL CONTENT, AND TOCOPHEROL CONTENT AND DISTRIBUTION IN
DURUM-WHEAT MILLED PRODUCTS

Sample	Mill yield	Oil	Tocopherol in 100 g		
			In oil	Mill product	Distribution
	%	%	%	mg	% of total
Semolina	53.7	0.75	0.034	0.255	16.5
First clear flour	4.3	1.52	0.034	0.517	2.6
Second clear flour	12.2	2.37	0.042	0.995	14.6
Red dog	10.1	4.55	0.038	1.729	21.1
Shorts	11.6	4.85	0.048	2.328	32.5
Bran	8.0	4.41	0.030	1.323	12.8
Durum wheat	—	2.07	0.052	1.076	—

TABLE V

TOCOPHEROL CONTENT AND BIOASSAY DATA UPON NONSAPONIFIABLE FRACTIONS
(Durum-wheat milled products)

Source	Tocopherol in sterol-reduced nonsaponifiable	Level fed	Bioassay	
			Tocopherol in sample fed	Total litter
	%	mg	mg	%
Whole wheat oil	2.33	42.8	1.0	57
Semolina oil	1.71	58.2	1.0	50
First clear oil	1.96	51.0	1.0	57
Second clear oil	2.49	40.0	1.0	100
Red dog oil	2.71	36.8	1.0	86
Shorts oil	2.00	50.0	1.0	71
Bran oil	1.30	77.0	1.0	57

of approximately 55% total litter efficiency found for four of the samples might be taken to indicate the presence of a tocopherol showing slightly less activity than pure alpha. Such a possibility is partly substantiated by the fact that the three samples showing the equivalent of full alpha activity are the second-clear, red-dog, and shorts oils in which the majority of the germ tocopherol would be concentrated. This would suggest that the nongerm tocopherol of durum wheat contains either a considerable proportion of beta- or a small content of gamma-tocopherol. It is impossible, however, upon the evidence available to make any definite statement on this point until more extensive data have been secured; the most that can be claimed with any degree of certainty is that the vitamin E of this particular wheat possesses a high biological activity approaching that of alpha-tocopherol.

The data given in Table IV indicate that the hard and durum wheats tested contain approximately equal amounts of vitamin E, but that the

distribution is somewhat different. Since the milling systems for the two wheats are dissimilar, no direct comparisons can be made. The semolina and patent flour fractions, however, should be roughly comparable as they both represent a close approximation to pure endosperm. It is therefore of interest to note that the semolina contains an appreciable quantity of the vitamin, amounting to 16.5% of the total, whereas patent flour is practically free. It does not appear probable that this sizable tocopherol content of semolina is due to germ contamination, since the oil contents of semolina and patent flour are practically identical.

In the durum milling system, no germ is secured and the tocopherol derived from the embryo must, therefore, be completely distributed over some of the other milled products. It is generally believed that the majority finds its way into the shorts, but the results obtained in this study suggest that a considerable amount goes into the bran, since this fraction is relatively high in both oil and tocopherol.

As with the hard wheat, computation indicates that the embryo cannot account for more than 50% to 60% of the total tocopherol found in the wheat. The principal difference between the two types of wheat appears to reside in the distribution of this " nongerm " tocopherol. In durum it seems to be fairly uniformly distributed throughout the endosperm, whereas in hard wheat it is localized in the layers closely adjacent to the bran.

It should be again pointed out that these results with durum wheat are representative of the mix and procedure employed in a particular mill and must, therefore, be regarded as indicative only until confirmed or disproved by more extensive studies.

Summary

The historical background, isolation, and chemical and physiological characteristics of the tocopherols are briefly reviewed and discussed.

Difficulties involved in the biological assay of materials containing low concentrations of the vitamin are described and the unsuitability of existing analytical procedures pointed out.

Reference is made to and a brief outline given of a method developed for the chemical analysis of such products as the oils extracted from milling products of cereal grains. This method involves chromatographic separation of the nitric acid oxidation products upon activated alumina.

Biological assay data are presented which indicate that the preparatory steps employed do not result in measurable loss of tocopherol and also that the results obtained by the chemical method correlate well with those secured by animal assay.

Analyses of milled products derived from hard and durum wheats are presented.   These indicate that both wheats contain essentially similar amounts of vitamin E and suggest that this is present substantially as alpha-tocopherol.   The distribution within the kernel differs, however. In both wheats, about 55% is located in the embryo and in the hard wheat the remainder is found in the endosperm layers closely adjacent to the bran, this latter tissue together with the bulk of the endosperm constituting patent flour being practically free from the vitamin.   In durum wheat, the nongerm tocopherol is fairly uniformly distributed throughout the endosperm, the semolina containing appreciable amounts.

Acknowledgments

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THE APPLICATION OF THE THIOCHROME METHOD TO THE THIAMIN ANALYSIS OF CEREALS AND CEREAL PRODUCTS [1]

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(Read at the Annual Meeting, May 1941)

For a period of more than forty years since the pioneer work of Eijkman in 1897 the vitamin B_1 content of cereals and cereal products has been of considerable interest to an ever-increasing number of investigators. From the early observations that whole rice grain would prevent the beriberi that resulted from dietaries largely comprised of polished rice came a recognition that cereals constitute an important class of foods aside from their content of starch, protein, and minerals. It is now generally agreed that cereal grains are one of the most important dietary sources of vitamin B_1 or thiamin.

The early work on this subject was necessarily slow, partly because of the absence of adequate analytical methods. The use of fowls and later rats as subjects for assay left much to be desired. These methods were expensive and time-consuming and available only to a limited number of specially equipped laboratories.

Today the picture has been decidedly changed by the development of microbiological and chemical procedures. No longer is it necessary to make a considerable investment of money for a vitamin B_1 analysis that may be completed a month or two after the sample has been submitted for assay. Results can now be obtained within a few hours and in some instances within a few minutes at a modest cost.

One of these rapid methods is the thiochrome procedure. In 1935 Peters discovered that the oxidation of thiamin resulted in its conversion

to a strongly blue fluorescent substance which was soon identified and named thiochrome. In the following year Jansen (1936) utilized this property of the vitamin as the basis of an analytical method. The vitamin was oxidized, extracted with isobutanol, and the fluorescence of the extract compared to that of a standard quinine solution. Two years later Kinnersley and Peters (1938) reported that this method might in some instances fail to record the full vitamin activity. They found that the cause was the presence of cocarboxylase, the pyrophosphate ester of thiamin. This substance possesses the equivalent physiological potency of the vitamin but the thiochrome oxidation product is insoluble in isobutanol and thus escapes detection. This difficulty was overcome by Hennessy and Cerecedo (1939) by enzymatically converting cocarboxylase to thiamin during the extraction of the sample. These investigators also introduced the use of zeolite as an efficient and convenient means of separating the vitamin from impurities which interfere in its determination.

For the past two and one-half years the thiochrome method has been employed in this laboratory for evaluating the thiamin content of cereals and cereal products. During this period a large number of samples have been examined and minor modifications of the method have been introduced for greater operating convenience. Some of the observations made during these investigations are being reported here.

Comparison of Thiochrome and Bioassay Methods

Hennessy and Cerecedo (1939) have compared the results obtained from their modification of the thiochrome method with those obtained by bioassay. Their list includes several samples of cereals and in all cases excellent agreement was obtained by the two types of methods. These comparisons have been extended by including a wider variety of cereal products and determining the thiamin content by both the thiochrome and rat-growth methods. The assay values are given in Table I.

In most instances the results from the two methods are in very satisfactory agreement and in no single sample is the discrepancy sufficiently great to throw serious doubt on the validity of the thiochrome values. The bioassay method is susceptible to an experimental error which somewhat limits its value as a comparison standard. In the list (Table I) some of the bioassays were made without any knowledge of the thiamin content. Accordingly, the levels chosen for the assay required guesswork. This accounts for some of the qualifying statements about the actual rat-growth values. In other cases the samples were first analyzed by the thiochrome method. Bioassay levels chosen from these values have never failed to give growths comparable to those from the thiamin standard.

TABLE I

COMPARISON OF THIAMIN VALUES OBTAINED BY RAT GROWTH AND
THIOCHROME METHODS

Sample	Thiamin in micrograms per gram	
	By rat growth	By thiochrome
	μg per gram	μg per gram
Wheat germ	30	31.5
Red dog flour	Slightly less than 15	15.1
Fortified cereal I	Slightly less than 10.5	9.7
Wheat cereal I	More than 7.5	7.9
Wheat cereal II	6	6.3
Fortified cereal II	Less than 6.6	6.1
Long extraction flour I	6	6.0
Wheat cereal III	6	5.8
Oat meal	4.8	5.4
Fortified flour	Slightly less than 5.2	5.4
Whole wheat flour	Between 4.5 and 5.4	5.1
Long extraction flour II	Less than 2.4	1.8

Discussion of the Thiochrome Method

The amount of sample taken for the assay is determined largely by its thiamin content. It is preferable to use a quantity which will contain approximately 9 μg or 3 International Units. This quantity has been chosen in order to have about 1 μg in the 5 ml of purified extract taken for oxidation; and also be similar in this respect to the standard thiamin solution which is simultaneously carried through the whole procedure. This use of the standard solution is desirable since its analysis serves as a check on the various operations and counteracts the effect of small errors arising from such steps as filtration and the base exchange in the zeolite purification.

In the analysis of low-potency materials such as patent flour a compromise must be made since it is difficult to extract the desired quantity. Four to five grams is used with 50 ml of the solvent and a larger aliquot is passed through the zeolite to bring the final concentration as near as conveniently possible to the desired 1 μg per 5 ml.

The use of a quantity of sample which will supply 9 μg represents the ideal. For practical purposes a considerable variation from this figure will give satisfactory results. As Hennessy and Cerecedo (1939) have pointed out, the base exchange is efficient over a considerable range. If the sample contains between 5 and 15 μg of thiamin, satisfactory results can be obtained without changing the amounts of solutions used in the zeolite purification.

For the extraction the originally proposed acetic acid solvent has been used. Other acids will serve satisfactorily, since their use is pri-

marily designed to stabilize the thiamin during the extraction.[2]   If mineral acids are employed, subsequent partial neutralization must be effected with a buffer salt; with acetic acid, caustic will produce the desired buffer action.

Figure 1 is a photograph of the extraction equipment employed.   It is merely a steam heated water bath designed for six simultaneous ex-
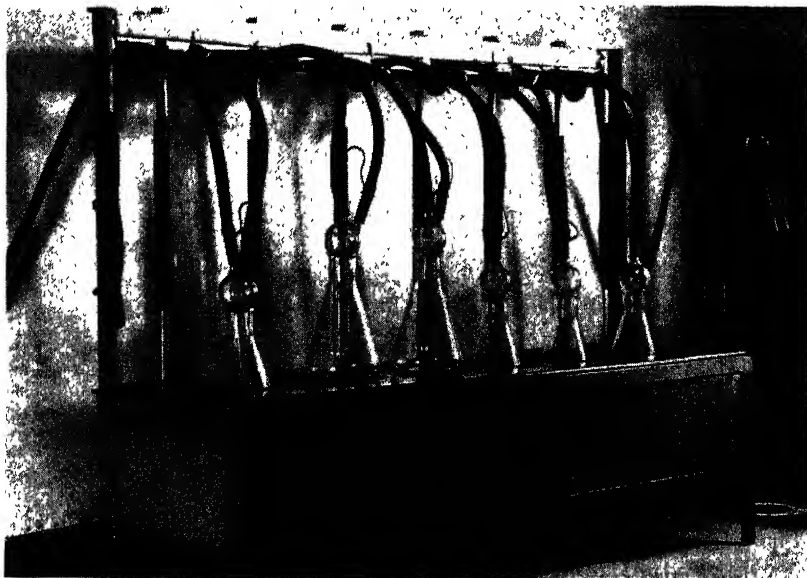
Fig. 1.   Extraction apparatus.

tractions.   The extraction vessel is a 250-cc Erlenmeyer flask and the condensers are of the " cold finger " type.   They possess the advantage of being inexpensive and easy to use.   They require no ground joints or stoppers which tend to stick or contaminate the extract.   Figure 2 is a sketch showing the condenser construction.   Cold water flows through the interior to keep the outer condensing surface cooled.

The next step involves enzymatic hydrolysis with a preparation of diastase.   Either the originally proposed takadiastase or the preparation known as Clarase is satisfactory (Proceedings of Vitamin Assay Conference, 1940).   The enzymatic hydrolysis has two functions: first, it breaks down the starch, facilitating extraction of the thiamin and subsequent clarification of the extract; second, it converts any cocarboxylase

[2] Recent observations suggest that more drastic extraction conditions may be required in the analysis of bread.   One sample which was extracted by autoclaving with 0.1N H₂SO₄ for 20 minutes at 15 lbs pressure gave a 10% higher value for thiamin than when extraction was carried out by refluxing with acetic acid.   Other samples of bread, however, have given the same values by both extraction methods.   Where autoclaving is used the sulfuric acid solvent is preferred to acetic acid.

into free thiamin.   With most cereals and cereal products the latter action is not particularly important since the quantities of cocarboxylase present are negligibly small.   It has been shown, however, that wheat germ is an exception and must be hydrolyzed before oxidation to the thiochrome.   Bread is another and even more important exception.   In
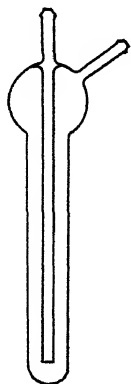
Fig. 2.   " Cold finger " type of condenser.

some of the early analyses of this type of product serious discrepancies were observed.   Low values and poor checks from replicate determinations were obtained.   This difficulty was removed only after raising the temperature of the enzymatic hydrolysis or prolonging the digestion period considerably.   It seemed probable that some reaction other than rapid starch hydrolysis was required for reliable analytical results.   Since the hydrolysis of cocarboxylase is relatively slow the difficulties were attributed to the presence of this thiamin derivative.   However, the flour from which the bread was prepared contained no cocarboxylase and it was necessary to assume that phosphorylation of the thiamin occurred during the yeast fermentation of the dough.   A preliminary examination of this possibility was made by examining at various intervals of the thiamin content of a suspension of flour, thiamin, and yeast.   Table II shows the results.

When hydrolysis with takadiastase was used for preparing the samples for assay there was no significant change in the thiamin during fermentation.   When takadiastase was omitted, the amount of free thiamin decreased.   After three hours nearly one-third of the original thiamin could not be measured without the use of the enzyme.   While this experiment does not prove that thiamin is converted to cocarboxylase during fermentation it does demonstrate the importance of thorough enzymatic hydrolysis in the analysis of bread.

TABLE II
The Thiochrome Analysis of Flour-Thiamin-Yeast Suspensions
(Thiamin in micrograms per gram of flour)

	Results from thiochrome analysis	
Fermentation time	With takadiastase	Without takadiastase
	µg per gram	*µg per gram*
2 minutes	4.98	4.89
30 minutes	4.80	3.72
1 hour	4.89	3.42
3 hours	4.62	3.24

The next steps involve clarification and base exchange. For clarification either centrifugation or filtration may be used. Centrifugation has the advantage of preventing the possible loss of thiamin from the extract by adsorption which may occur on filter paper. On the other hand, clarification is less complete and as a consequence the flow of the extract through the zeolite is retarded. While this is a minor item there is a tendency for the insoluble material to deposit on the zeolite particles and more rapidly lower the efficiency of the base exchange when the zeolite is used for a number of successive determinations. Filtration has been found to be satisfactory and convenient. A small loss of thiamin apparently takes place but this is entirely compensated for by treating the standard thiamin solution in the same manner. It is possible, however, that different types of filter paper may vary. They should be checked by comparing the thiamin contents of two aliquots of the standard, one of which has been filtered.

For the base exchange, tubes similar to those described by Hennessy [3] at the 1939 Convention of the American Association of Cereal Chemists have been used. Figure 3 shows a photograph of a series of these tubes ready for operation. One operator can easily handle 12 to 15 zeolite purifications simultaneously.

Keeping in mind that the base exchange step was introduced as a means of eliminating interfering impurities it was of interest to determine how the analyses of cereals would be affected if this operation was omitted from the procedure.

Several experiments have been carried out in which the filtered extract was oxidized directly. Table III compares the results obtained with and without the zeolite treatment.

In no single instance was there more than 5% difference between the values from the two methods. In nearly all the samples the agreement was as good as can be obtained from replicate assays by the regular thio-

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[3] Unpublished.

Fig. 3.   The zeolite purification.

TABLE III

COMPARISON OF THE THIOCHROME VALUES OBTAINED WITH AND WITHOUT THE
ZEOLITE PURIFICATION

	Thiamin in micrograms per gram	
Sample	With zeolite	Without zeolite
	μg per gram	μg per gram
Enriched flour	4.23	4.20
Patent flour	0.81	.84
Corn meal	1.74	1.71
Low grade flour	5.70	5.49
Cereal I	6.57	6.63
Cereal II	4.41	4.20

chrome procedure.   At least in the samples presented in this table it is believed that the amounts of impurities which carry through the determination and affect the fluorescence readings are negligible.   When the zeolite is omitted care must be taken that the isobutanol solution of the thiochrome is entirely clear.   A greater tendency to cloudiness will be observed, but this can be overcome by using larger amounts of finely ground sodium sulfate followed by brief centrifuging.

To determine whether or not the method could be simplified further the extraction process has been examined.   A few experiments have been tried by simply shaking the sample with a 25% potassium chloride

solution in dilute acetic acid, filtering, oxidizing, and measuring the fluorescence of the isobutanol extract. Table IV compares the results thus obtained with those given by the regular procedure.

TABLE IV

COMPARISON OF THE THIAMIN VALUES OBTAINED BY THE REGULAR THIOCHROME PROCEDURE AND BY OXIDATION OF KCl EXTRACTS

. Sample	Regular thiochrome method	Oxidation of KCl extracts	Differ- ence
	μg per gram	μg per gram	%
1 Enriched flour	3.90	3.93	− 0.8
2 Patent flour	0.75	0.69	− 8.0
3 Low grade flour I	8.64	8.58	− 0.7
4 Whole wheat flour I	4.26	4.38	+ 2.8
5 Low grade flour II	5.70	6.00	+ 5.3
6 Low grade flour III	30.9	30.6	− 1.0
7 Whole wheat flour II	5.1	4.59	−10.0
8 Red dog	15.0	13.6	− 9.3
9 Rice bran	37.5	34.2	− 8.8
10 Rice polishings	30.0	27.6	− 8.0
11 ˙Bread (air dried)	3.75	1.83	−51.0

In only one instance was there an outstanding difference between the results from the two methods. This was the sample of bread where the potassium chloride extract contained less than 50% of the amount obtained by the regular thiochrome method. Such a result would be expected in view of the necessity for enzymatic hydrolysis to liberate all the vitamin as free thiamin.

Of the first six samples of flour only the patent showed a material percentage difference. It is believed that this can be attributed to the very low thiamin content since the 8% difference observed between the two methods is also frequently observed in replicate analyses by the complete thiochrome method.

Samples 7, 8, 9, and 10 gave 8% to 10% lower values by direct oxidation of the potassium chloride extracts. Whether this difference can be removed by some modification of the extraction procedure, or whether diastasis is required to evaluate the total thiamin content, remains to be determined. In either event the relatively high efficiency of extraction by merely shaking with a salt solution offers considerable promise and is being further studied.

Especially in the case of enriched flours the use of this procedure should prove to be of material value. Not only is all the added thiamin extracted but also practically all of the vitamin naturally present in the flour. Assay results are in essential agreement with those obtained by the regular thiochrome method and since the complete analysis can be

done in a comparatively short time the procedure is well adapted for purposes of controlling products.

Experimental

The thiochrome method used in the present studies is that described by the American Association of Cereal Chemists (1941). For the analyses of flours by extraction with potassium chloride solution the following procedure was employed:

Two and one-half grams of " enriched " flour was placed in a 250-ml Erlenmeyer flask and carefully suspended in 50 ml of 25% potassium chloride solution in 2% acetic acid. The suspension was shaken intermittently over a period of 15 minutes, then filtered, and 5 ml of the filtrate was oxidized and its fluorescence determined as in the regular thiochrome procedure. A standard solution containing 10 μg of pure thiamin chloride in 50 ml of the 25% potassium chloride-2% acetic acid solution was similarly filtered and a 5-ml portion of the filtrate oxidized and its thiochrome content determined. " Blanks " were determined for the unknown and standard and these values deducted from the fluorescence readings of their corresponding oxidized solutions. The " corrected " value for the standard is the fluorescence of 1 μg of thiamin and by simple proportionation the value for the unknown was determined.

Example: The fluorescence of an isobutanol extract of 5 ml of the unknown solution was 27.5 divisions. The blank was 3. Fluorescence of the standard was 27.5 with a blank of 2.5. Thus,

$$27.5 - 3/27.5 - 2.5 \times 50/5 \times 1/2.5 = 3.92 \ \mu g/gram.$$

Summary

The thiamin contents of a variety of cereals have been determined by both rat growth and thiochrome assays. The results obtained by these two methods are in essential agreement. Operating details of the thiochrome procedure are discussed and abbreviated techniques applicable to certain types of cereal products are reported.

Acknowledgments

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QUICK TEMPERING OF WHEAT FOR EXPERIMENTAL MILLING

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It has always been a disadvantage to wait overnight for wheat samples to temper before milling. This is particularly true during the harvest when grain buyers are especially anxious to learn as quickly as possible the milling and baking characteristics of new wheat samples.

For over a year we have used a surface-active agent in our tempering water whenever we have wanted rapid results.   In the past we were forced to temper our hard spring and winter wheats from 8 to 18 hours or overnight in order to secure the best milling results. By use of a wetting agent we have been able to cut our tempering time from overnight to two to three hours.

Our procedure is to do a moisture test on the wheat, then clean and weigh 2,000 g of the sample. A table is made indicating the correct number of milliliters to be added to a given weight of wheat at any moisture content to bring the moisture level to 15.0%. The sample is shaken thoroughly with the added water in an air-tight, screw-top glass container and when tempered sufficiently long it is milled.

For quick tempering we merely substitute approximately 0.1% solution of Aerosol OT (sodium dioctyl sulfosuccinate) in place of the tap water ordinarily used for tempering. This wetting agent is made by the American Cyanamid and Chemical Corporation. No doubt there are many other surface-active agents which might give equally good results.

Some wheats which are particularly difficult to temper may require four to five hours for the best results. The optimum time for the

wheats handled by the laboratory in any given crop year can be deter-
mined with a few trial experiments, using a tempering time from two
to five hours with the solution of the wetting agent as compared with the
overnight procedure with water on the same wheat. If, for any reason,
the sample tempered with the wetting agent has to stand for a longer
period of time the results are entirely satisfactory.

With the amounts of the particular surface-active agent used, we
have noticed no difference in the milling yield, ash, protein, mixing
curve, or baking results of the flours as compared with the flours milled
by the usual long tempering method. The saving of time with the
resultant speed in obtaining milling and baking results is sometimes a
great asset in purchasing wheat.

BOOK REVIEW

Cereal Laboratory Methods, Fourth Edition. Compiled by Committee on Revision, American Association of Cereal Chemists, 110 Experiment Station Hall, College of Agriculture, Lincoln, Nebraska, 1941. Price $2.50.

The fourth edition of *Cereal Laboratory Methods* bears abundant witness to the rapid strides that have been made in the field of cereal chemistry since the publication of the first compilation of methods nearly 20 years ago. Even the third edition, which seemed like a rather imposing volume when it was published in 1935, seems slender and inadequate in comparison with this new edition. In spite of the fact that the size of the present volume has been considerably reduced through the use of charts instead of tables for conversion of ash and protein to different moisture levels, the fourth edition is still half again as large as the 1935 volume.

Recent progress in the development of new methods and the improvement of older methods for testing the baking quality of various types of flour is especially evident. All these baking test methods have been rewritten and amplified in accordance with the recommendations of the various committees of the A. A. C. C. The methods as they now stand are more clearly presented than in the past, and useful information is included on the interpretation of results and the descriptive terms used in scoring the baked products. This new edition makes it more than ever apparent that the cereal chemist has developed distinctive methods for testing the materials that are produced and utilized in the plants in which he works. A large number of the methods given in this book are taken from the files of *Cereal Chemistry* and there is less dependence than heretofore upon methods from other sources.

Turning first to the new material, the book contains four new chapters dealing with Experimental Milling, Rye, Malt, and Experimental Macaroni Processing. Chapter II, on experimental milling, will be welcomed by many as it gives recommendations and specifications for the milling equipment used for both bread wheats and durum wheats, as well as flow sheets for experimental milling. This is probably as comprehensive and yet compact a treatise on experimental milling as has ever appeared. Chapter V, on rye, includes two methods for detection of ergot, six methods for the determination of rate of gelatinization, and methods for the determination of lactic, acetic, and butyric acids. Chapter VI, on malt, gives methods for physical and chemical testing of malt. Among the chemical methods are determinations of extract, color of wort, total wort nitrogen, diastatic power, alpha-amylase, and proteolytic activity. Chapter X, on experimental macaroni processing, gives directions for production of disks for color measurement, and outlines the process of manufacturing macaroni on experimental equipment. This is supplemented by new material in Chapter XI, dealing with the determination of color and cooking characteristics of alimentary pastes.

For the first time the terms thus far defined by the Committee on Definitions of Technical Terms have been included in a book of methods. These terms are given in Appendix A as a glossary of cereal chemical terms. An innovation which will be helpful to cereal chemists is the excellent treatise on statistical principles and experimental errors which constitutes Appendix B. This treatise will fill a long-felt need for a brief and easily understood discussion of the applications of statistical principles to the analysis of cereal chemical data. Among the statistics and procedures explained are variance, standard deviation, analysis of variance, the t test, and the correlation coefficient. Included in Appendix B is an interesting compilation of experimental errors in the chemical and physical determinations commonly employed in cereal laboratories. In each case the standard error of a single determination is given.

Appendix C, on preparation and standardization of solutions, will be appreciated by every cereal chemist. Instructions are given for the common acids and alkalies and a number of salt solutions and buffer solutions.

The charts for correcting ash and protein to either the 13.5% or 15.0% moisture basis are convenient and compact. These four charts take the place of 72 pages of tables. If these charts were to be sold separately they would undoubtedly find wide acceptance among cereal chemists.

These new chapters, appendices, and charts, however, are by no means the only changes in the book, as revisions and additions have been made throughout. To mention some of the more important changes, to Chapter III, on flour and semolina, have been added sections on the determination of thiamin by the thiochrome and

fermentation methods and the qualitative and quantitative determination of iron. The Snell and Strong method for riboflavin is recommended but not described.

Chapter IV, on feeds and feeding stuffs, has been supplemented with methods for solids in syrups and molasses, fat in dried milk products, lactose in mixed feed, and methods for determination of phosphorus, manganese, chlorine and iodine.

Additional methods for the determination of staleness in bread are given in Chapter IX. To Chapter XII has been added a simple method for the determination of the specific volume of creams, cake batters, and icings. Chapter XIII, on yeast foods and flour improvers, has a new section on bleaching agents made up of methods formerly included in the chapter on flour and semolina and embracing new qualitative and semiquantitative methods for benzoyl peroxide.

The tables at the end of the book have been amplified, making the cereal chemist less dependent upon his chemist's handbook.

CLINTON L. BROOKE

FLOUR BLEACHING WITH CHLORINE DIOXIDE [1]

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(Read at the Annual Meeting, May 1941)

The use of chlorine dioxide for flour bleaching was discussed by Staudt (1928) in a British patent in which improvements in flour baking characteristics and color removal similar to that achieved by the use of nitrogen trichloride were reported. A treatment of one or two grams of chlorine dioxide per 100 kilograms of flour was recommended.

Another patent was granted to Becher (1933) in which claims were made covering the use of combination bleaching treatments involving chlorine dioxide with nitrogen oxides, nitrosyl chloride, and organic peroxides. The authors have encountered nothing in the scientific literature describing the use of chlorine dioxide as a flour bleaching agent.

Despite the good bleaching results possible with chlorine dioxide, little interest has been shown in the reagent by flour millers, perhaps because no cheap and reliable method has been available for producing the gas. However, by 1935 the Mathieson Alkali Works, Inc., developed means for producing chlorine dioxide economically from a technical grade of sodium chlorite which they manufacture. Chlorine dioxide can conveniently and economically be produced from technical sodium chlorite either by the electrolytic process of Logan (1939) or by the solution process of Cunningham and Losch (1936).

Experimental

In the present studies on the treatment of flour with chlorine dioxide, the electrolytic method of Logan (1939) was used for generating the gas. This method consists essentially in the electrolysis of a solution containing about 144 g per liter of $NaClO_2$ and 140 g per liter of NaCl

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in a cell in which the anode and cathode compartments are separated by a porous diaphragm. Suitable materials for the electrodes are graphite, nickel, and copper.

The solution is fed continuously into the anode compartment and after electrolysis is disposed of through an overflow in the cathode compartment. Chlorine dioxide is formed at the anode and is stripped from the solution by a stream of air which keeps the partial pressure of the chlorine dioxide below explosive levels. The partial pressure of the chlorine dioxide in the chlorine dioxide-air mixture may be further reduced by dilution with more air after leaving the cell. The chlorine dioxide-air mixture is then led into an agitator suitable for flour bleaching. Chlorine dioxide obtained by this process is substantially free from chlorine. Control of the chlorine dioxide output is effected through control of the current. Approximately an hour must be allowed for the cell to reach equilibrium, during which time the solution feed, current, and air flow for stripping off the chlorine dioxide are carefully controlled and maintained at constant rates. Each cell must be calibrated for chlorine dioxide output with different values of current, solution feed, and air flow. For batch bleaching purposes for the treatment of a few pounds of flour, it is convenient to calibrate a cell at one current value, one air flow (for stripping), and one feed flow. The dosages on the flour may then be regulated by using the constant output of the cell for varying lengths of time.

Chlorine dioxide reacts practically instantaneously with flour as do the other gaseous reagents in common use, such as chlorine, nitrogen trichloride, and nitrogen peroxide.

It was found in general that less chlorine dioxide is needed to bring about optimum bread-baking characteristics in flour than is the case with nitrogen trichloride. From 0.3 to 1.5 g of chlorine dioxide per barrel of flour is usually sufficient to achieve the same maturing action that can be secured with about twice as much nitrogen trichloride. It is essential, therefore, to control accurately the amount of chlorine dioxide applied to flour in order to avoid overtreatment.

Chlorine dioxide is very efficient as a color-removing or bleaching agent for flour. Used at the maximum rate that is compatible with optimum baking characteristics, it will usually oxidize more carotinoid pigments than the optimum amount of nitrogen trichloride applied to the same flour. In fact, it will often bleach flour satisfactorily without the aid of any other bleaching agent, whereas compositions containing benzoyl peroxide are usually used to supplement bleaching treatments with nitrogen trichloride. Figure 1 shows typical examples of the relationship between the amount of chlorine dioxide applied and residual coloring matter in a patent and a clear flour.

It is evident from the curves in Figure 1 that the greatest color removal with chlorine dioxide occurs with the first increments of the gas applied to a patent flour.   However, in the case of a first clear flour, a substantial amount of chlorine dioxide must be applied before much color removal results.   At this writing, commercially bleached patent flours usually contain between 1.3 and 0.6 ppm of carotinoid pigments (expressed as " carotene ") determined by the naphtha-alcohol extraction procedure.   Figure 1 shows that these " carotene " levels are reached when 0.6 to 1.4 g of chlorine dioxide per barrel of flour is applied to the
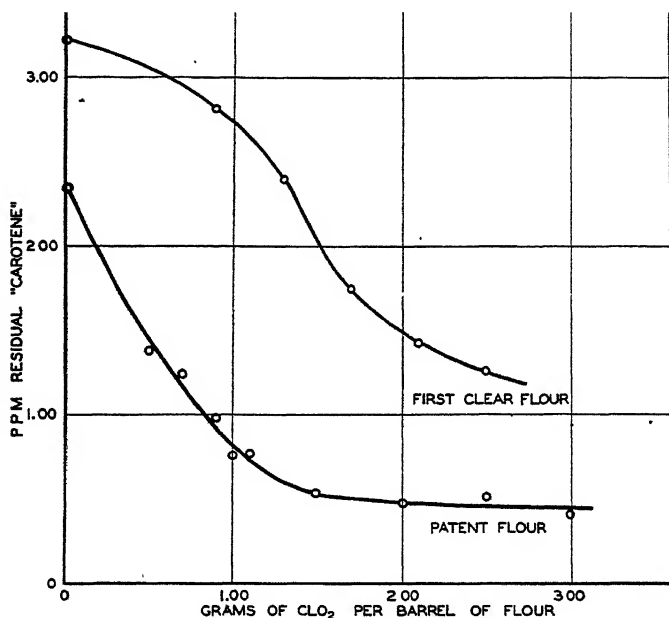
Fig. 1.   Carotinoid pigment content of chlorine dioxide bleached flours—naphtha-alcohol extraction basis.

patent flour in question.   Experiments with many patent and clear flours have demonstrated that the shape of the curves shown in Figure 1 is characteristic for each type of flour.

With most reagents used for flour bleaching, there is a correlation between flour slick color and bread crumb color.   In the case of chlorine dioxide, however, this correlation is not found consistently.   There appears to be some tendency towards dullness in the slick score of flours bleached with this reagent, but no corresponding dullness is found in the bread crumb color.   In fact some very white crumb colors with no trace of dullness have been found in bread made from flours which produced

quite dull slicks, showing that in the case of this reagent, the slick does not have its usual significance, bread crumb color being the best and most significant criterion. Of course when flour is overbleached with chlorine dioxide, the crumb color will become dull just as in the case of over-bleaching with any other reagent.

Chlorine dioxide exerts a maturing action on flour similar to that produced by nitrogen trichloride. Frequently the grain and loaf volume of bread are improved by chlorine dioxide treatment. While similar improvements are achieved in most cases by nitrogen trichloride, instances have been encountered in which either one or the other reagent is the more effective. Chlorine dioxide affects the dough-handling properties of some flours, producing a dryer, easier handling dough. Overbleaching with chlorine dioxide produces a " bucky " type of dough.

TABLE I

CHLORINE DIOXIDE BLEACHED PATENT FLOUR—COLOR AND BAKING CHARACTERISTICS [1]

Bleaching treatment per barrel of flour	Carotinoid pigments [2]	Slick score	Absorption	Crumb		Loaf volume
				Color	Grain	
	ppm					*ml*
Unbleached	2.92	Yellow	64	Yellow	10	2740
Mill bleached flour	1.29	10	64	10	10	2795
0.5 g ClO_2	1.71	9 creamy	65	8 creamy bright	10	2830
0.8 g ClO_2	1.18	10 slightly dull	66	11 bright creamy white	10	2815
1.5 g ClO_2	0.75	8 dull	66	13 white	10	2685

All other baking characteristics were the same for the mill bleached and chlorine dioxide bleached flours.

[1] Commercial formula, straight-dough procedure. One-pound loaves. Three hours' fermentation.
[2] Expressed as carotene. Naphtha-alcohol extract.

Some of the color and baking characteristics of chlorine dioxide bleached flours are shown by way of example in Tables I and II for patent and first clear flours respectively. In Table I it is seen that the optimum slick score is achieved at a dosage of 0.8 g of chlorine dioxide per barrel of flour, a greater treatment causing a decrease in the slick score. However, the crumb color of the bread from patent flour becomes whiter as the dosage of chlorine dioxide is increased to 1.5 g per barrel of flour. The color removal becomes greater, of course, as the chlorine dioxide dosage is increased. It can be seen in Table I that the optimum treatment from the standpoint of loaf volume is less than 1.5 g per barrel. While more closely graduated dosages would indicate

the optimum treatment more accurately, the data in the tables illustrate typical trends.

Table II illustrates the color and baking characteristics of a first clear flour treated with varying dosages of chlorine dioxide. In this case the slick score increases until a treatment with 2.1 g of chlorine dioxide per barrel of flour is reached and then at 2.5 g per barrel it falls off rapidly. Just as with the patent flour, the crumb color score increases as the chlorine dioxide treatment of the flour is increased. In this case no appreciable falling off in volume occurs with chlorine dioxide treatments up to 2.5 g per barrel. It can be seen that the grain is improved by both nitrogen trichloride and chlorine dioxide treatments, but only 1.7 g of chlorine dioxide is needed to bring about as much improvement in the grain as 5 g of nitrogen trichloride per barrel of flour; further, 1.7 g of

TABLE II
CHLORINE DIOXIDE BLEACHED FIRST CLEAR FLOUR—COLOR AND BAKING CHARACTERISTICS [1]

Bleaching treatment per barrel of flour	Carotenoid pigments [2]	Slick score	Crumb		Loaf volume
			Color	Grain	
	ppm				ml
Unbleached	2.64	Yellow	Yellow	7 spherical harsh	585
5 g NCl₃ (control)	1.50	10	10	10	630
0.9 g ClO₂	2.32	7 creamy	6C	8 spherical	610
1.3 g ClO₂	1.96	9 creamy	9C	9 spherical	595
1.7 g ClO₂	1.34	11	11	10	620
2.1 g ClO₂	1.05	12	11+	10	620
2.5 g ClO₂	0.93	7 dull	12	10	615

All other baking characteristics were the same for both the chlorine dioxide and nitrogen trichloride treated flours and the unbleached flour.

[1] Basic A.A.C.C. formula plus 0.25% Arkady.
[2] Expressed as carotene. Naphtha-alcohol extract.

chlorine dioxide in this instance has given better color removal than 5 g of nitrogen trichloride, as evidenced by the " carotene " and crumb color figures given in Table II. The data illustrate the greater effectiveness of unit dosages of chlorine dioxide. Presumably chlorine dioxide is a more potent reagent than nitrogen trichloride for flour bleaching purposes.

Chlorine dioxide has very little effect on the pH of· flour in the amounts in which it is used for bleaching. From this standpoint it is not a substitute for chlorine. The effect of chlorine dioxide on pH is usually less than that of a nitrogen trichloride and benzoyl peroxide combination

bleaching treatment, due in part, no doubt, to the fact that much less chlorine dioxide is needed to treat flour than nitrogen trichloride.

Chlorine dioxide alone has been found capable in some instances of substituting for commercial bleaching treatments such as nitrogen trichloride and benzoyl peroxide, nitrogen trichloride alone, benzoyl peroxide alone, or chlorine and benzoyl peroxide.

In order to preserve the keeping qualities of flours treated with chlorine dioxide, it has been found desirable to apply less than 2 g per barrel of this reagent. If sufficient color removal cannot be obtained with the application of 2 g or less of chlorine dioxide, then benzoyl peroxide may be used to achieve additional color removal.

Summary

Chlorine dioxide is an effective flour bleaching and maturing agent. Compared with other commercial bleaching agents, smaller dosages in terms of grams per barrel are required; hence its application requires accurate control. As anticipated, clear flours require heavier dosages than patent flours.

When chlorine dioxide is used in the amount needed to give optimum baking characteristics, it usually removes more color than the optimum quantity of nitrogen trichloride on the same flour. Grain and loaf volume usually reflect the maturing action of chlorine dioxide. Dough-handling properties are affected sometimes, a dryer feeling dough being obtained. The pH of flour is not affected by the quantity of chlorine dioxide used in flour bleaching. For optimum keeping quality, the dosage should be kept preferably below 2 g per barrel of flour.

Acknowledgment

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EFFECTS OF MOISTURE ON THE PHYSICAL AND OTHER PROPERTIES OF WHEAT

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(Received for publication March 14, 1941)

Wheat, to be of best quality, as measured by test weight and color, should not receive any added moisture after it is dry-ripe. When wheat contains 30% to 35% moisture, it is mature enough to harvest with a binder without shriveling. Further physical changes in the wheat are due mostly to desiccation. When wheat is to be harvested with the combine, the moisture content should not be more than 14%, preferably less. If wheat is wetted by rain after it is dry-ripe more or less change occurs, the extent depending on the amount of rain and the prolongation of wet weather. Showers followed by sunshine are not as damaging as prolonged drizzles with much cloudy weather. However, even small showers followed by sunshine may affect color and test weight in such a manner as to influence the grain grading properties.

Since test weight is an important factor in grain grading, its being lowered by wetting after the wheat is dry-ripe causes large losses to the wheat growers. Fifty-one-pound test weight wheat, badly bleached because of prolonged rains after it was ready for the combine, has been observed, yet this wheat appeared plump, and the indications were that the flour yield would not be lowered in proportion to the decrease in test weight.

Previous Investigations

The percentage of yellowberry was not influenced in samples of wheat cut at various stages of maturity and then subjected to various conditions of wetting and drying (Swanson, 1936). It was indicated that yellowberry was caused by conditions existing prior to the cutting of the wheat. The test weight was lowered, the amount depending upon the number and duration of the wetting periods. Diastatic activity was influenced only when the amount of wetting was sufficient to stimulate the process of germination (Swanson, 1935).

Mangels and Sanderson (1925) found a high positive correlation in each crop year between average test weight and average flour yield. Bailey (1923) reported differences in flour yield of 2.5% from different lots of 60-pound wheat and 3.6% from 58-pound wheat.

Bracken and Bailey (1928) harvested and threshed wheat just as soon as the wheat was ripe and repeated at ten-day intervals, six cuttings

and threshings, covering 50 days.   They found that three rains totalling 0.69 inch completely bleached the wheat and reduced the test weight four pounds per bushel, due to an increase in kernel volume and a decrease in density.   Kernel sections were shown to be opaque.   Subsequent rains did not produce further decrease in test weight.

Sharp (1927) found that "after the moisture content of the once air-dried wheat has been increased and the moisture again removed, the wheat does not regain its former density, the decrease depending on the amount of moisture taken up.   When corneous kernels were increased in moisture content to between 18% and 25% and the moisture reduced again by drying, the resulting kernels were opaque and when cut were invariably starchy in appearance.   The decrease in density is due to the formation of air spaces in the kernel after the removal of the water."

Whitcomb and Johnson (1930) threshed wheat at monthly intervals at Bozeman, Montana, from shocks exposed to the weather from August until the following March.   The prevailing weather in the fall was dry and the winter was unusually cold.   "Test weight per bushel showed a greater change as weathering progressed than did any other property of the wheat considered."   For Marquis the maximum decrease in test weight was 5.2 pounds and for Kanred, 6 pounds.   "The loss in test weight was due to decrease in specific gravity caused by the swelling of the kernels when they became wet and their failing to assume normal size when they dried out."   Whitcomb and Johnson observed that the dark kernels decreased from 86% to 17% for Marquis, and from 72% to 34% for Kanred.

Relation of Flour Yield to Test Weight

Differences in reports on the correlation or lack of correlation between test weight and flour yield arise in part from a failure to distinguish between test weight as influenced by shape of kernel caused by weather conditions during ripening or by factors of inheritance, and test weight as influenced by moisture conditions after the wheat is dry-ripe.   The percentage of endosperm in the kernel increases constantly during the process of development (Bailey, 1925).   If the wheat is cut before endosperm development is completed or if the full development is prevented by very hot and dry weather, the percentage of endosperm will be decreased and as a consequence the flour yield will be lowered. The test weight of such wheat, because it is correlated with plumpness, will be a good criterion of the probable flour yield.   That variety is also a factor is shown by the generally higher test weights of Blackhull and Chiefkan as compared with Kanred and Tenmarq.   It has not been satisfactorily demonstrated that the flour yields of the two former varie-

ties are correspondingly higher. However, when the test weight is lowered because of kernel swelling and roughening of the bran coat due to wetting, no change in the proportion of endosperm and bran occurs, and the flour yield is not decreased in proportion to the lowered test weight.

The swelling of the kernels, due to wetting, disorganizes the more dense original structure and, hence, increases the internal air space. The scattering reflection of light by these irregularly distributed and variously shaped air capillaries causes the opaque or mealy appearance, similarly as the innumerable air bubbles give the white color to the Easter lily.

Outline of the Experiments

The object of the experiments reported in this paper was to determine the effects of wetting and drying wheat, both before and after threshing, on several factors used in evaluating quality. The wetting and drying before threshing had two phases: (1) wetting bundles from one to four times and then drying, one set in the sun and another in the shade; and (2) exposing wheat in the field in four shocks for about two months. The wetting and drying of the threshed grain was done in the laboratory as will be described later. The measurements for quality made on these samples were as follows:

1. Test weight was determined by the official method.

2. Color, texture and general appearance of the grain were obtained by two methods: (1) Envelope samples were submitted to Martin Shuler of the Kansas City office of the Grain Inspection Department for official grading. (2) A barley kernel cutter was used to cut 50 kernels into halves, exposing the cut sections for observation.

3. Milling tests were made to obtain flour for other measurements and to determine to what extent flour yield was influenced by changes in the test weight.

4. Pearling tests were made to determine hardness of the grain.

5. The diastatic activity was determined on all the samples treated in the straw and on a few of those treated in the grain. No effects in the latter were obtained.

6. Baking tests on selected samples were made to determine the effects of the various treatments on loaf volumes and bread textures.

7. The carotene content was determined on selected samples representing the extremes, but the amounts obtained were not correlated with the treatments.

8. Dough-mixer curves were made on a few representative samples.

Turkey wheat from a uniform field was used.[1] No rain fell during the 1940 harvest from the time the wheat was maturing until the latter part of July, except for a trace on June 23. Hence, wheat was obtained both in bundles and as threshed grain which had not been appreciably wetted during ripening or after desiccation in the field.

Treatment of unthreshed wheat.[2]—Enough wheat was cut on June 22 to furnish bundles for four large shocks and for wetting and drying in the shade and sun. The wheat was in a condition fit to cut with the binder, the straw was all yellow, and the grain was in the very hard dough stage.

One shock was covered with a water-proofed canvas securely anchored; another was capped with bundles securely tied so as not to blow off; a third shock had no cover or cap bundles; and the fourth was built like the third, but from this bundles were taken and threshed at various intervals as follows: July 1, July 24, July 29, August 13, and September 5. The first two of these threshings had had no rain except a trace on June 23. The rainfall during July and August was as follows:

July 27	0.29 inch	Aug. 16	0.60 inch
Aug. 8	1.35 inches	Aug. 17	0.40 inch
Aug. 13	0.30 inch	Aug. 22	0.06 inch
Aug. 14	0.75 inch	Aug. 26	0.72 inch
Aug. 15	0.31 inch	Aug. 27	0.38 inch

The wheat which was threshed on July 29 was wetted by one 0.29-inch rain; that threshed on August 12 received two rains amounting to 1.64 inches. Ten rains totaling 5.16 inches had wetted the shocks of the final threshing made September 5. At this threshing. the uncovered shock and the shock capped with bundles were each divided into two portions, outside and inside bundles, but the ·canvas-covered shock was not divided. The remaining bundles from the fourth shock were threshed as the final sample.

The bundles for the wetting and drying in the sun and shade were divided into two groups, and separate bundles in each group were wetted according to the following scheme:

<div style="text-align:center">*Times Wetted*</div>

	Times Wetted	
Group 1:	0, 1, 2, 3, 4	Dried in the shade after each wetting.
Group 2:	0, 1, 2, 3, 4,	Dried in the sun after each wetting.

The bundles were wetted by immersing the heads in water for a definite number of minutes, but for each group there was one lot that received no wetting. The first soaking was for 30 minutes, the second for 20

minutes, the third for 10 minutes, and the fourth for 5 minutes. The drying proceeded very slowly after the third and fourth soakings in the bundles dried in the shade and the straw turned dark. To hasten drying, the straw in each bundle was turned so that what was on the inside of the bundle came to be on the outside. No rain fell, but on some days the sky was overcast.

Each lot of the bundles dried in the sun were placed in the shade as soon as they were dry after the designated number of soakings. After the third wetting some sprouting was observed in some of the bundles dried in the shade. All these bundles were threshed July 8.

Treatment of the threshed wheat.—The wheat used for wetting as grain was cut July 1, and five bushels of grain threshed as soon as practicable. Since the last rain occurred on June 11, the wheat had not been wetted since it was ripe. The wheat was cleaned in the laboratory separator and then exposed in shallow layers so that the moisture content should become low and uniform. The moisture content of a composite sample was 10.3%, vacuum oven, and the test weight 61.3 pounds.

The general plan was to place 1800-gram portions in gallon bottles; add water to make the moisture percentages of the various portions to 12, 14, 16, 18, 20, 22, 24, 26, and 28%; shake well so as to distribute the water evenly; determine the test weights as soon as the water was absorbed; then expose in shallow paper boxes until dry; and then determine the test weight of the dried samples. The exposure to the dry laboratory air was continued for each sample until the weight was near 1800 grams. Tests showed that a few grams of variation had no significant influence on test weight. Wetting and drying were repeated from one to six times at the 2% moisture intervals from 12% to 28%, inclusive. Thus the wheats in the bottles of Lot 1 were wetted once, test weight taken in duplicate, then dried and test weight taken. This process was applied the same number of times as the lot numbers. Thus the wheat in Lot 2 was wetted twice and dried after each wetting; three times for Lot 3; four times for Lot 4; five times for Lot 5; and six times for Lot 6.

The dry, hot weather in July, 1940, favored the rate of drying after each wetting. As soon as the samples were dry, they were returned to the original bottles until the next treatment. A 1400-gram portion was taken from each of the dried samples for milling; an 8-ounce bottle for reserve; 75 grams for grading the grain; and the remainder was for use in determining hardness by means of a barley pearler, and also for estimating the counts of mealy, semivitreous, and vitreous kernels with the use of a barley kernel cutter.

Results of Treatments Before Threshing

The data obtained from the various treatments before threshing are given in Table I. The moisture content was low in the samples threshed before the rains started, but the percentages were higher in those from the shocks threshed the latter part of August and the first part of September. No rain had fallen between August 27 and September 5 when the last threshing was done. The wheat in the covered shock had 12.3% moisture. About 2% had been absorbed from the more humid atmosphere which prevailed during August. Atmospheric conditions also prevented the drying out of the wheat wetted by rains in the exposed shocks to the level that was obtained when the wheat was threshed during the very dry weather.

TABLE I

TREATMENTS: TEST WEIGHTS, MALTOSE VALUES, AND PERCENTAGES OF MOISTURE, FLOUR YIELD, AND FLOUR ASH ON SAMPLES TREATED BEFORE THRESHING

Treatment	Number wettings	Threshed	Moisture wheat	Test weight		Flour yield	Flour ash	Diastatic activity
				Cleaned	Scoured			
		date	%	lbs.	lbs.	%	%	mg. maltose
Bundles:								
Soaked and dried in sun.....	0	7/8	10.0	60.5	63.2	71.8	0.43	112
" " " " "	1	7/8	10.4	60.4	62.8	72.6	0.42	117
" " " " "	2	7/8	10.1	60.5	63.0	72.4	0.40	105
" " " " "	3	7/8	10.3	58.9	62.0	70.0	0.39	111
" " " " "	4	7/8	9.9	58.7	61.7	69.9	0.38	109
" " " " shade...	0	7/8	10.1	60.2	62.9	72.8	0.40	140
" " " " " ...	1	7/8	10.4	60.1	63.0	72.6	0.40	139
" " " " " ..	2	7/8	10.3	58.1	61.2	69.4	0.40	156
" " " " " ..	3	7/8	10.3	58.3	61.3	68.4	0.38	212
" " " " " ..	4	7/8	10.2	57.8	61.3	68.2	0.38	267
Exposed shocks:	Rain, in.							
Not capped:								
Sample 1................	0.00	7/1	10.0	60.3	62.9	72.6	0.46	135
" 2........	0.00	7/24	10.9	60.1	63.1	73.6	0.46	122
" 3........	0.29	7/29	10.9	59.1	62.3	73.6	0.42	115
" 4...	1.64	8/15	11.0	56.7	60.2	71.9	0.42	160
" 5.....	5.16	9/5	13.0	55.8	59.9	70.6	0.42	335
Covered.....	5.16	9/5	12.3	60.4	63.0	70.9	0.43	233
Capped—outside bundles....	5.16	9/5	12.5	57.3	61.2	69.1	0.41	264
Capped—inside bundle......	5.16	9/5	11.7	58.3	61.9	69.2	0.41	203
Not capped—outside bundles	5.16	9/5	13.3	55.1	59.6	69.2	0.42	330
Not capped—inside bundles..	5.16	9/5	13.3	58.2	62.1	70.3	0.40	258

Effect of test weight on flour yield.—The various wettings as well as the exposure to the rains in the shocks decreased the test weight. The least change was in the samples dried in the sun, in the wheat from the covered shock, and from the two threshings from the shock before the rains came. The samples wetted several times and dried in the shade and the samples taken from the outside of the shocks decreased the most in test weight. On some of these bundles there was evidence of sprout-

ing. The larger test weights obtained after scouring indicate that the decrease in test weights of the wetted as well as the weathered samples was in part due to the roughened condition of the bran coat. Scouring made the kernels more smooth and allowed closer packing and hence greater test weight.

The flour yields showed a trend toward a decrease as the test weight became less in the samples wetted and dried in the sun or in the shade. However, since the ash percentages also show a trend toward a decrease, it is a question whether the lower flour yield may not have been due to a lower extraction. The samples obtained from the exposed shocks showed little or no correlation between test weight and flour yield as well as between ash content and flour yield. ·Neither did the various treatments have any consistent effect on the ash percentages.

Diastatic activity.—There was no correlation between diastatic activity and treatments in the samples wetted and dried in the sun or in the samples from the shocks threshed before the rains came. There was a considerable increase in diastatic activity in the samples wetted and dried in the shade, especially those wetted three or four times. The greatest diastatic activity was found in the samples taken from the exposed portions of the shocks in which there was evidence of sprouted kernels. Diastatic activity is greatly increased by conditions of germination (Swanson, 1936). The fact that the sample from the covered shock also had a high diastatic activity indicates that moist weather conditions which prevailed through August had an effect in increasing the diastatic activity. Since this was evident in only one sample, effect of high humidity on diastatic activity needs further investigation.

Results of grain grading.—The official grading of these samples indicated the numerical grade, their subclass, and the percentages of vitreous and damaged kernels as shown in Table II. The test weight (Table I) was apparently the principal factor in determining the grade in all but two samples, which were given sample grade. These two were from the bundles most exposed in the shocks and both of these had large percentages of total damage. The percentages of vitreous kernels show a distinct trend toward a decrease corresponding with the amount of wetting and exposure. The trend toward decrease in vitreousness also correlated with the increase in total damage.

Texture and hardness tests.—A barley kernel cutter was used to examine the cross sections of wheat kernels. The cutter has 50 single-grain pockets which hold the wheat kernels while they are cut into halves by a moving steel cutting blade. After the instrument is opened the cut half sections of the 50 kernels are exposed so that they may be examined. The average results from two counts of mealy, semivitreous, and vit-

TABLE II

GRADING OF WHEAT SAMPLES TREATED BEFORE THRESHING

Treatment	Number wettings	Test weight cleaned [1]	Grade	Vitreous	Total damage
		lbs.		%	%
Bundles:					
Soaked and dried in sun	0	60.5	1 DHW	84	0
" " " " " . . .	1	60.4	1 DHW	83	0
" " " " "	2	60.5	1 DHW	72	0
" " " " "	3	58.9	2 DHW	75	0.4
" " " " "	4	58.7	2 HW	63	0
" " " " shade. . . .	0	60.2	1 HW	68	0
" " " " "	1	60.1	1 HW	73	0
" " " " "	2	58.1	2 HW	28	3.0
" " " " "	3	58.3	2 DHW	77	3.2
" " " " "	4	58.7	3 HW	64	2.2
Exposed shocks:					
Not capped	Rain, *in.*				
Sample 1	0.00	60.3	1 HW	70	0
" 2	0.00	60.1	1 DHW	87	0
" 3	0.29	59.1	2 DHW	75	0
" 4	1.64	56.7	3 HW	60	10.0
" 5	5.16	55.8	5 SGHW	28	16.4
Covered	5.16	60.4	1 HW	72	0
Capped—outside bundles	5.16	57.3	5 HW	33	11.8
Capped—inside bundles	5.16	58.3	2 HW	58	0.4
Not capped—outside bundles .	5.16	55.1	5 SGHW	40	20.0
Not capped—inside bundles . .	5.16	58.2	3 HW	63	6.8

[1] These test weights are repeated from the data in Table I.

reous kernels from the wheat samples treated before threshing are given in Table III.

Taylor, Bayles, and Fifield (1939) have developed a useful test for estimating the hardness of wheat. In our laboratory a machine similar to the barley pearlers used in grain inspection was employed. The samples before and after pearling were weighed on a balance sensitive to 0.01 g. and the losses computed in percentages.

The pearling losses for the samples dried in the shade were larger than for the samples dried in the sun, with one exception. The lowest losses for the shock samples were obtained from the covered shock and from the one sample threshed before the rains started. The largest losses were thus obtained from the samples exposed the most, demonstrating that exposure to moisture caused softening of the kernels.

The wetting and drying of the wheat in the sun and shade did not have as great an effect as when exposed to rain in the shocks. The wetting period was apparently not long enough to soak the kernels, and it was mostly the outside surface that was affected. Roughening of the

TABLE III

Treatment	Number wettings	Mealy	Semi-vitreous	Vitreous	Loss in pearling
		%	%	%	%
Check...........	—	4	2	94	—
Bundles:					
Soaked and dried in sun	0	4	2	94	32.4
" " " " "	1	4	4	92	33.4
" " " " "	2	2	4	94	32.9
" " " " "	3	10	8	82	36.2
" " " " "	4	10	22	68	34.9
" " " " shade....	0	10	12	78	34.7
" " " " "	1	8	2	90	33.9
" " " " "	2	4	4	92	34.5
" " " " "	3	2	6	92	31.9
" " " " "	4	4	10	86	38.5
Exposed shocks:					
Not capped:	Rain, *in.*				
Sample 1...............	0.00	10	6	82	37.6
" 2........	0.00	2	10	84	34.8
" 3................'	0.29	4	10	86	39.5
" 4................	1.64	30	20	50	40.3
" 5....	5.16	40	24	30	41.1
Covered...................	5.16	8	8	84	33.6
Capped—outside bundles....	5.16	58	24	18	38.0
Capped—inside bundles......	5.16	26	22	52	36.6
Not capped—outside bundles.	5.16	42	22	36	40.4
Not capped—inside bundles..	5.16	34	18	48	36.4

bran surface lowered the test weights. Exposure in the shocks showed the greater effect of the rain, and the wheat from the more exposed bundles from the outside of the shocks had a much larger increase in the mealy kernels (or a decrease in the vitreous kernels) than wheat from the inside bundles which were more protected. Capping the shocks with bundles was not very effective in preventing the grain from becoming wet as the results from the capped and the uncapped shocks were not widely different. Covering with canvas, which entirely prevented wetting, was highly effective in preventing the development of the mealy condition. In the uncapped shock from which samples were periodically threshed, there was no increase in the mealy kernels before the rains came or with the 0.29-inch rain. The rains following produced a marked increase in the number of mealy kernels.

Results of Treatments After Threshing

Effect of amount of wetting on test weight.—The test weights on the wet, the dry, and the scoured samples that were wetted and dried after

threshing are given in Table IV. The largest decrease in test weight in proportion to the amount of water added took place in increasing the moisture from the original moisture of about 10.3% to 12% or 14%. Wetting beyond 14% decreased the test weights proportionately much less, and the decreases were still smaller in wetting above 20% and 22% moisture. It appears that at 20% and 22% moisture, nearly the maximum amount of swelling had taken place, since the changes produced beyond this were comparatively small.

Effects of number of wettings on test weight.—A single wetting decreased the test weight proportionately more than the subsequent wettings. But while the decreases in test weights for the first wetting were larger than for each of the subsequent wettings, the trend toward the decrease persisted up to the sixth or last wetting, especially for the dried and scoured samples. This can be seen by noting the total decreases (bottom row, Table IV) which were obtained by subtracting the test weights obtained from the samples wetted to 28% from the check figures. While the largest amount of kernel swelling occurred with the first wettings, the subsequent wettings produced additional amounts of swelling.

Causes of decrease in test weight.—The fact that the decrease in test weight persisted after the wetted samples had been dried to the original moisture content shows that after the structural arrangements of the interior of the wheat kernels have been disturbed by addition of water, the original compactness cannot be restored by drying to the original moisture content. The decrease in test weight of wheat wetted one or more times is due partly to roughening of the bran coat and partly to increase in the volume of the wheat kernels and not to any loss of material. An examination of cross sections of kernels from samples wetted the most showed that the vitreous condition present in the original wheat had been replaced by a chalky or mealy texture. When the endosperm contracts after swelling by water the starch cells and other substances remain in a more or less disarranged condition, leaving numerous air spaces throughout the endosperm. The reflection of light from a mealy endosperm is similar to that from snow, and the reflection of light from a vitreous kernel similar to that from ice.

The influence of the condition of the bran coat on the test weights is shown by the larger test weights of the scoured samples. These larger test weights were due to closer packing in the test kettle because of smoother kernel surface. The trends toward decreases in test weights in the scoured samples as a result of wetting and drying are similar to trends in the unscoured dried samples. That is, the decreases are proportionately greater for the smaller amounts of wetting and also for the one wetting as compared with the following wettings. This is similar

to the results obtained by Bracken and Bailey (1928) in which it was shown that the first rains produced the greatest decreases in test weight.

TABLE IV

TEST WEIGHTS AS AFFECTED BY WETTING AND DRYING

	WET					
Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
% Check	61.3	61.3	61.3	61.3	61.3	61.3
12	59.6	59.3	59.2	59.0	59.2	58.6
14	58.1	57.9	57.6	57.6	57.8	57.1
16	57.3	56.6	56.4	56.4	56.0	55.5
18	55.8	55.1	54.3	54.2	54.1	54.7
20	54.0	53.3	52.9	52.6	53.2	52.1
22	51.9	51.6	51.1	50.8	51.8	51.5
24	50.6	50.6	50.0	50.0	50.0	50.5
26	49.8	49.8	49.0	48.9	49.0	48.9
28	49.7	49.2	48.5	48.7	48.8	48.9
Total decrease	11.6	12.1	12.8	12.6	12.5	12.4
% Check	61.3	61.3	61.3	61.3	61.3	61.3
12	60.1	59.5	59.3	59.2	59.3	59.0
14	58.5	58.5	58.4	58.3	58.1	57.7
16	58.1	57.5	57.8	57.6	57.1	57.1
18	57.7	57.1	57.1	56.9	56.4	56.7
20	57.3	56.5	56.9	56.8	55.8	55.7
22	57.1	56.5	56.4	56.3	55.8	55.6
24	56.8	56.3	56.1	56.1	55.5	55.5
26	56.8	57.0	56.0	55.8	55.4	55.5
28	56.5	56.1	55.9	55.6	55.3	54.7
Total decrease	4.8	5.2	5.4	5.7	6.0	6.6
% Check	64.3	64.0	63.5	63.4	63.5	63.5
12	63.3	63.0	62.8	62.3	62.6	62.0
14	62.2	62.1	61.9	62.2	61.7	61.0
16	62.0	61.2	61.7	61.1	61.0	60.3
18	61.6	61.1	61.1	60.4	60.3	59.7
20	61.5	60.6	60.5	60.2	59.8	59.5
22	61.4	60.5	60.4	59.9	59.6	59.1
24	61.1	60.4	60.3	59.7	59.6	59.0
26	61.1	61.0	60.2	59.6	59.5	59.0
28	60.8	60.2	60.0	59.4	59.4	58.2
Total decrease	3.5	3.8	3.5	4.0	4.1	5.3

Milling Results

The milling was done on the Buhler mill with a constant roll and feed setting. The proper mill settings were determined by making a number of millings on check samples. Fourteen hundred grams of each sample were milled. The amount of tempering water was calculated on the basis of the moisture content of the samples, which was found to be near 10%, varying ¼% above or below. At first it was assumed that tempering to 16% moisture would be best for the checks as well as for those which had been wetted the lesser amounts, and that those which had been wetted to the larger amounts should be tempered to less than 16%. A few trials, however, showed that those which had been wetted to the ·larger amounts also milled best when tempered to 16%, and hence this was the amount used for nearly all the samples. The tempering was done in two stages. All but 14 cc. of water was added the evening before milling and the last 14 cc. was added about 15 minutes before starting to mill. Because the milling room is air conditioned, it was possible to hold the relative humidity near 70% and the temperature near 80°F.

TABLE V
BREAK FLOUR FROM WETTED WHEATS

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%	%	%	%	%	%	%
Check	12.9	12.6	13.0	12.8	12.6	13.3
12	12.4	12.2	13.2	12.7	12.3	13.2
14	13.2	12.1	12.0	12.8	12.1	13.0
16	12.2	12.5	12.0	12.8	12.5	13.1
18	12.6	12.3	12.4	13.1	12.9	13.8
20	13.8	13.9	13.0	14.5	13.4	15.3
22	13.3	13.9	13.9	14.9	14.9	15.9
24	13.8	14.1	14.1	15.0	14.8	15.4
26	13.9	13.6	14.2	14.6	14.2	·14.7
28	14 1	14.4	14.7	14.1	14.4	13.8

Percentages of break flour.—As a general rule, more break flour is obtained from soft than from hard wheats. To determine whether these samples had softened by the water treatments, the break flours were weighed separately, and their tabulated percentages are given in Table V. The figures show a small trend towards larger percentages of break flour with the increased amounts and number of wettings, which indicates some softening but not as much as might be expected.

The percentages of total flour.—The percentages of total flour were calculated by dividing the total weights of flour obtained by the 1400 g.

of wheat used, and the data from the various samples are given in Table VI. This method of calculation involves a small error since the tempering water contributes to the amount of flour. Part of the tempering water goes to the bran and shorts and part to the flour. There is also the so-called " invisible loss " which in most cases amounted to about half the weight of the tempering water. However, since results from all the samples were treated the same, the figures are comparable.

There is no definite trend towards increase or decrease in the percentages of flour obtained as a result of the amount or number of wettings. The total decreases in test weight (bottom row, Table IV), of the dry, unscoured wheat was 4.8, 5.2, 5.4, 5.7, 6.0, and 6.6 pounds respectively for the number of times wetted. The flour yields, computed on the weight basis, were just as large for the samples whose test weights had been decreased from 4.8 to 6.6 pounds as for the check samples (Table VI). The evidence shows that the flour yield of wheat was not affected by the number of times the wheat had been subjected to wetting. This was due to the fact that the various wettings had not caused any loss in weight but only increased the space occupied by the

TABLE VI

TOTAL FLOUR FROM WETTED WHEATS

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%	%	%	%	%	%	%
Check	73.9	73.0	74.6	74.7	75.3	74.6
12	73.2	73.2	76.3	75.0	73.8	74.6
14	74.0	74.5	73.0	74.8	74.0	74.6
16	73.0	72.2	73.0	74.3	74.0	72.6
18	72.0	72.6	72.2	73.0	73.6	74.3
20	76.1	75.7	73.0	75.2	72.5	74.3
22	75.0	73.7	73.1	74.8	76.1	76.0
24	73.5	73.0	74.9	75.4	75.0	76.2
26	75.0	73.6	74.0	75.4	75.3	75.2
28	73.6	73.8	75.0	75.2	76.3	75.4

kernels. A given volume of kernels will weigh less after wetting, but a given weight of kernels had the same proportion of endosperm from which the flour is made as before the decrease in test weight by wetting.

Ash content.—Flour yields must be considered in connection with their ash contents. Since the setting of the rolls will influence yield, this will also affect the ash percentage. But as has been mentioned, these samples were all milled with a constant roll and feed setting. Percentages of flour ash (Table VII) apparently do not show any definite or significant trends. Hence, the lack of correlation of the test weights

TABLE VII

ASH OF FLOURS FROM WETTED WHEATS
(15% moisture basis)

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%	%	%	%	%	%	%
Check	0.43	—	—	—	—	—
12	0.43	0.39	0.46	0.41	0.41	0.43
14	0.42	0.41	0.44	0.41	0.41	0.43
16	0.40	0.43	0.44	0.42	0.40	0.43
18	0.42	0.43	0.40	0.42	0.41	0.44
20	0.42	0.41	0.43	0.40	0.41	0.42
22	0.41	0.40	0.42	0.43	0.44	0.42
24	0.43	0.41	0.42	0.43	0.41	0.43
26	0.40	0.43	0.41	0.44	0.43	0.43
28	0.43	0.45	0.41	0.43	0.44	0.41

(Table IV) with the flour yields (Table VI) are not due to nonuniform milling. Test weight gives the pounds of wheat in a given volume, while flour yield is computed in percent on the basis of weight, not volume.

Flour moisture.—The moisture percentages of the straight flours were fairly uniform as should be expected because the wheat was all tempered to the same moisture content and milled under uniform humidity and temperature conditions.

Tests for Hardness

A visual inspection of these samples showed that there was a definite trend towards decrease in the vitreousness of the samples or an increase in the mealiness, corresponding with the amounts and times of wetting. The pearling test, described in connection with the samples wetted before threshing, was made on all the samples of grain wetted to various percentages from one to six times. The results of these pearling tests are given in Table VIII.

TABLE VIII

LOSSES IN PEARLING INDICATING RELATIVE HARDNESS

Percentage to which wetted	Number of wettings					
	1	2	3	4	5	6
%	%	%	%	%	%	%
12	32.8	36.6	34.2	34.6	34.8	35.9
14	34.5	36.9	37.1	36.4	38.5	40.0
16	36.5	38.9	38.2	38.6	40.6	42.3
18	37.4	39.0	40.5	43.8	45.7	45.1
20	38.4	40.8	44.0	44.8	45.4	46.5
22	39.3	40.2	44.6	44.3	45.4	46.1
24	39.6	41.6	44.4	44.4	44.3	42.7
26	38.5	39.6	43.8	41.4	40.7	41.2
28	37.9	39.5	40.9	38.9	39.1	38.0

There was a definite trend in the increase of pearling losses from the 12% wetting up to the 20%–24% wettings, but beyond this there was a reversal in the trend. The reason for this is not entirely apparent. There was also a trend, but less regular, toward increase of pearling losses with the number of wettings.

The data on test weights (Table IV) show a decrease with the increase in amount of wetting as well as the number of wettings, but that the rate of decrease in test weight became less after the 20%–22% wettings, and also after the first wetting. There is a fair correlation between the decreases in test weight and the increases in the pearling losses.

Grades of Wheats That Were Wetted and Dried

The official grades, the percentages of dark hard vitreous kernels, and the percentages of total damage obtained by the U. S. Grain Inspection Department, Kansas City, Mo., are given in Table IX. The lowering of numerical grades corresponds closely with the decreases in test weights given in Table IV. There is also a distinct trend in changing from dark hard winter to hard winter with the amount and number of wettings. The percentages of dark hard vitreous kernels decreased very markedly with the increase in amount of wetting and to a much less extent with the number of wettings. The amount of wetting had a greater effect in decreasing the vitreous condition than the number of wettings. The reason for this has already been discussed.

Texture of Wetted and Dried Samples

The barley cutter, used on the samples treated before threshing, was used also on the samples wetted after threshing. The results of this examination are given in Table X, which gives the percentages of mealy, semivitreous, and vitreous kernels. Those kernels not definitely mealy or vitreous were designated as semivitreous.

The percentages of mealy kernels increased with the amount of wetting and with the number of wettings, and the vitreous kernels decreased similarly. The most abrupt change was at about 20% moisture. Moisture percentages lower than this have much less effect in increasing the number of mealy kernels or in decreasing the number of vitreous. The semivitreous also show a sharp increase from the 20% wetting and above. It is apparent, therefore, that in order to produce the mealy or semivitreous condition it is necessary to add enough water to produce a certain amount of swelling. To get this swelling at 20% moisture it is necessary to have more than one wetting. With several wettings the swelling that produces the mealy conditions is obtained in wetting to 20% moisture.

TABLE IX

MARKET GRADES OF WETTED AND DRIED WHEATS

	GRADES					
Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%						
12	1 DHW	2 DHW	2 DHW	2 DHW	2 DHW	2 DHW
14	2 DHW	2 DHW	2 DHW	2 DHW	2 DHW	3 DHW
16	2 DHW	3 HW	3 DHW	3 DHW	3 DHW	3 DHW
18	3 DHW	3 DH	3 HW	3 DHW	3 DHW	3 HW
20	3 DHW	3 DHW	3 HW	3 DHW	4 DHW	4 HW
22	3 DHW	3 HW	3 HW	3 DHW	4 DHW	4 HW
24	3 HW	3 HW	3 HW	4 HW	4 HW	4 HW
26	3 DHW	3 HW	3 HW	4 HW	4 HW	5 HW
28	3 HW	3 HW	4 HW	4 HW	4 HW	5 HW

Check   1 DHW, TW 61.3

	DARK HARD VITREOUS					
%	%	%	%	%	%	%
12	92	91	97	94	98	87
14	84	87	99	96	97	95
16	88	67	84	87	96	89
18	84	87	70	88	94	70
20	81	79	73	90	77	60
22	80	28	64	76	78	60
24	65	28	27	29	42	27
26	75	46	26	28	40	27
28	27	38	49	30	27	55

Check   DHV 88

	TOTAL DAMAGE					
%	%	%	%	%	%	%
12	0	3.0	0	0	0	0
14	0	0	1.5	0	0	0
16	3.0	3.3	0	0	1.0	0
18	1.4	2.4	1.5	1.0	0	0
20	2.3	2.0	5.0	3.0	0	5.0
22	3.8	2.5	6.0	3.0	2.0	0.7
24	0	5.2	3.0	4.2	10.0	10.0
26	0	2.0	3.0	3.5	9.4	11.0
28	0	8.0	6.7	4.3	10.0	15.0

Check   total damage 0

TABLE X

PERCENTAGES OF MEALY, SEMIVITREOUS, AND VITREOUS KERNELS IN FIFTY

MEALY						
Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%	%	%	%	%	%	%
Check	0	—	—	—	—	—
12	4	6	4	2	4	4
14	2	2	8	4	4	0
16	4	14	4	6	4	6
18	8	10	10	10	12	14
20	12	12	18	30	24	40
22	18	24	26	36	40	34
24	24	26	36	44	52	42
26	24	36	48	44	60	58
28	34	46	54	66	66	50

SEMIVITREOUS						
%	%	%	%	%	%	%
Check	2	—	—	—	—	—
12	8	2	4	4	4	2
14	8	6	4	6	6	10
16	8	36	4	4	8	14
18	10	44	16	28	34	28
20	50	54	46	52	42	46
22	52	56	48	48	44	48
24	54	64	46	44	38	44
26	68	22	38	46	30	34
28	56	46	38	32	28	38

VITREOUS						
%	%	%	%	%	%	%
Check	98	—	—	—	—	—
12	88	92	92	94	92	90
14	90	92	88	90	90	90
16	88	56	92	90	88	80
18	82	46	74	62	54	58
20	38	34	36	18	34	14
22	30	20	26	16	16	18
24	22	10	19	12	10	14
26	8	42	14	10	10	8
28	10	8	8	2	6	12

Baking Tests on Selected Samples

Baking tests were made on a few samples representing the various conditions of treatment both before and after threshing as indicated by the headings in Table XI, which also give the description of the samples selected for these tests and the results by two different formulas. These formulas were:

Formula I	Formula II

Formula I
100 g. flour
Absorption as needed
  5 g. sugar
  1 g. salt
  3 g. yeast
  0.3 g. malt syrup, 200 Lintner
  0.1 g. $NH_4H_2PO_4$
  2 mg. $KBrO_3$

Formula II
100 g. flour
Absorption as needed
  6 g. sugar
  1½ g. salt
  3 g. shortening
  2 g. yeast
  4 g. dry milk solids
  ¼ g. malt syrup, 120 Lintner
  3 mg. $KBrO_3$

A third formula which was also used differed from the second only in having 4 mg. $KBrO_3$ instead of three. The results were almost duplicates of those obtained from Formula II.

The doughs were mixed to the optimum as determined by observation in the Swanson-Working type of mixer. This time varied from 3¼ minutes to 4 minutes (84 r.p.m.). The three-hour standard fermentation and proofing times were used, namely 105 minutes to first punch, 50 minutes to second punch, 25 minutes to pan. The proofing time was 55 minutes, and the baking time was 25 minutes at 430°F.[3]

The choice of the comparatively rich formulas containing milk and several milligrams of $KBrO_3$ were based on a study of baking methods for evaluating the quality of hard red winter wheat varieties (Finney and Barmore, 1939). Twenty-two different methods were included in that study.

The mixing time obtained for Formula I was constant at 3¼ minutes for all the samples. The slightly longer mixing time for Formula II was probably due in part to the presence of the shortening. However, the sample exposed to the ten rains, totaling 5.16 inches, and the one wetted six times to 28% had the longest mixing times.

The figures for loaf volume are apparently of the most significance in arriving at the baking values provided they are accompanied by satisfactory grain, texture, and crumb color. These latter do not differ significantly among the different samples and hence these characteristics were unaffected by the various treatments.

The largest loaf volumes and the highest baking scores were obtained from the sample threshed from the uncovered shock exposed to ten rains

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[3] The baking tests were made by Karl F. Finney of the Hard Winter Wheat Quality Laboratory, to whom credit for this work is due.

TABLE XI

BAKING TESTS ON SELECTED SAMPLES

FORMULA I

Treatment	Mix. time	Abs.	Loaf vol.	Grain	Texture	Crumb color	Baking value	Number loaf Fig. 1
	min.	%	*cc.*	%			%	
Before threshing:								
Check	3¼	59	700	83	G+VG¹	63 cy²	80	1
No wetting, dried in sun	3¼	58	670	82	G+VG	65 cy	77	2
Wetted 4 times, dried in shade	3¼	60	703	82	G+VG	70 cy	81	3
Shock, canvas covered	3¼	58	683	82	G+VG	63 cy	78	4
Shock, threshed before the rains	3¼	61	723	82	G+VG	67 cy	82	5
Shock, threshed after ten rains	3¼	60	803	83	G+VG	65 cg	90	6

After threshing:								Number loaf Fig. 2
Times wetted — Per cent wetted								
1 — 12	3¼	59	710	82	G+VG	68 cg	81	1
1 — 28	3¼	62	740	82	G+VG	70 cy	85	2
3 — 22	3¼	60	710	82	G+VG	68 cy	81	3
5 — 22	3¼	61	680	82	G+VG	68 cy	78	4
6 — 12	3½	59	713	83	G+VG	65 cy	82	5
6 — 28	3¼	61	690	83	G+VG	70 cy	80	6

FORMULA II

Treatment	Mix. time	Loaf vol.	Grain	Texture	Crumb color	Baking value	Number loaf Fig. 1
	min.	*cc.*	%			%	
Before threshing:							
Check	3½	748	94	VG	82 cr²	91	7
No wetting, dried in sun	3½	725	93	VG	82 cr	89	8
Wetted 4 times, dried in shade	3½	768	94	VG	86 crg	93	9
Shock, canvas covered	3¼	755	90	VG	77 cr	85	10
Shock, threshed before the rains	3¼	783	90	VG	84 cg	94	11
Shock, threshed after ten rains	4	995	93	VG	88 cw	117	12

After threshing:							Number loaf Fig. 2
Times wetted — Per cent wetted							
1 — 12	3½	753	93	VG	83 cr	91	7
1 — 28	3½	805	92	VG	87 cg	97	8
3 — 22	3½	790	94	VG	84 cr	96	9
5 — 22	3½	778	94	VG	84 cr	94	10
6 — 12	3½	765	94	VG	84 cr	93	11
6 — 28	4	863	94	VG	85 cr	104	12

¹ G = good. VG = very good.
² cy = creamy yellow; cg = creamy gray; cr = creamy; cw = creamy white.

totaling 5.16 inches and from the sample wetted as grain six times to 28% and dried between wettings, except with Formula I; the latter sample gave no better values than the other samples wetted as grain. For all the other samples the loaf volumes and the baking values were not significantly correlated with the treatments either as grain or before threshing. Larger loaf volumes were obtained from all samples with Formula II than from Formula I.

Photographs of the loaves baked with Formulas I and II are shown in Figures 1 and 2. Figure 1 shows the loaves from the samples treated before threshing, and Figure 2 shows those from the samples wetted as grain. Loaves 1 to 6 in each figure were baked by Formula I and loaves
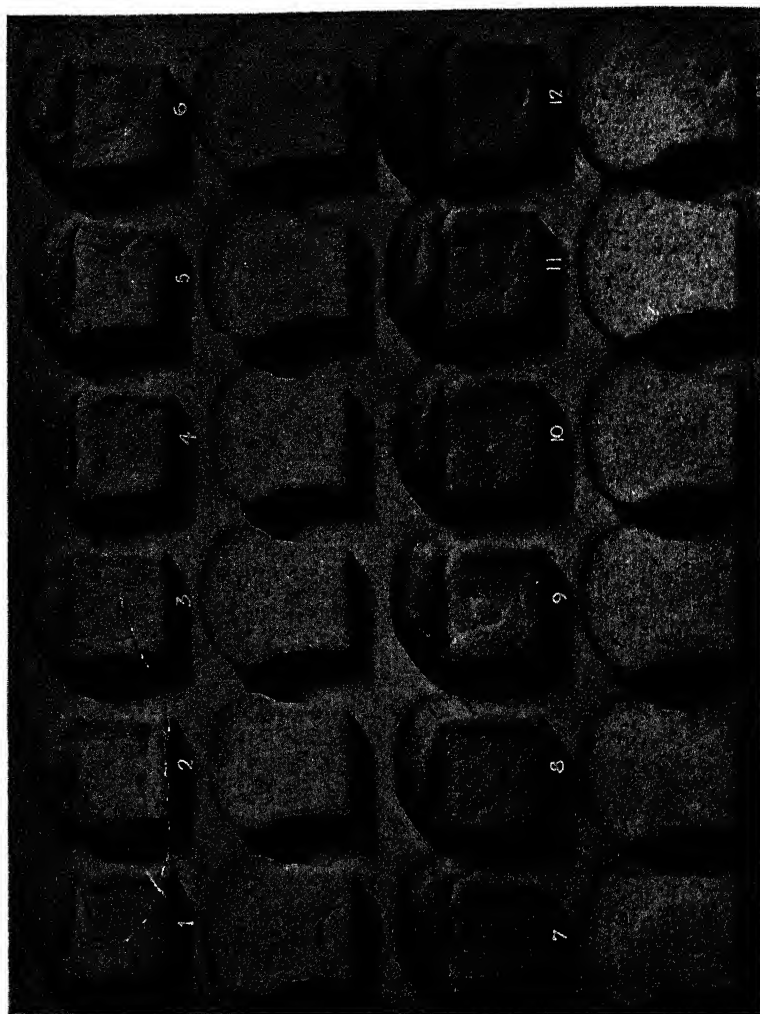
Fig. 1. Samples treated before threshing.

Loaf numbers 1 & 7, checks; 2 & 8, no wetting, dried in sun; 3 & 9, wetted 4 times, dried in shade; 4 & 10, canvas covered shock; 5 & 11, threshed from shock before rain; 6 & 12, threshed from shock after ten rains.

Loaves 1 to 6 baked by Formula I, 7 to 12 by Formula II.
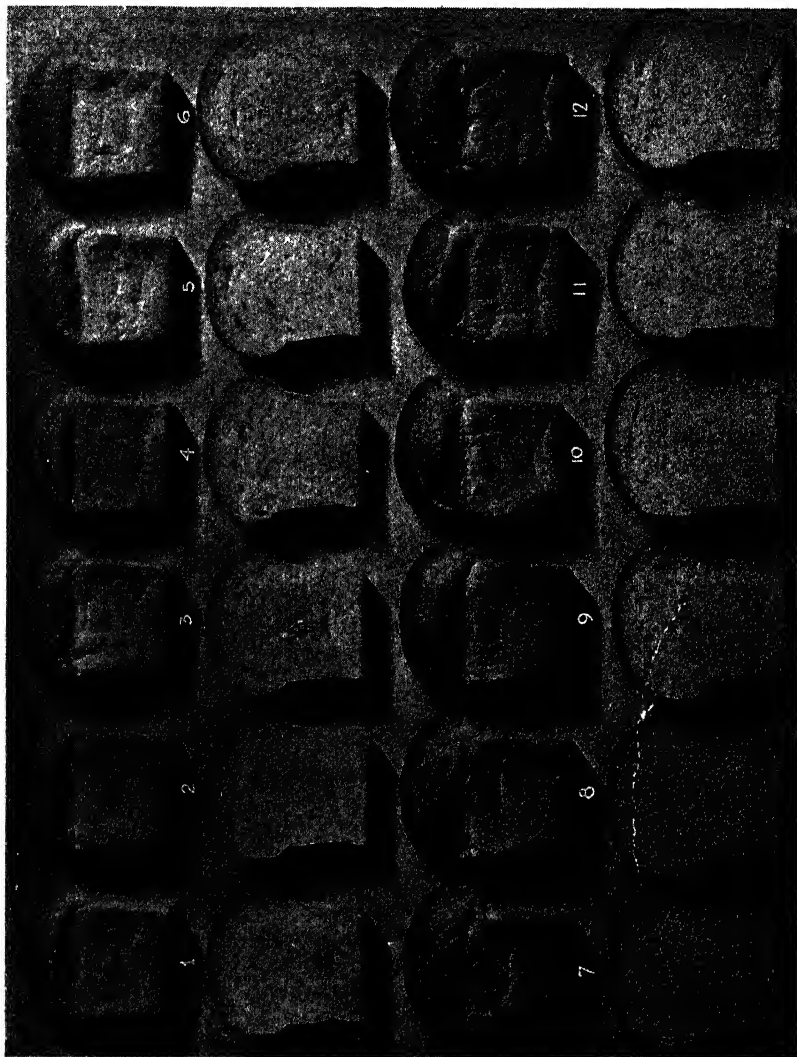
Fig. 2. Samples treated after threshing.

Loaf numbers 1 & 7, wetted once to 12%; 2 & 8, once to 28%; 3 & 9, three times to 22%; 4 & 10, 5 times to 22%; 5 & 11, 6 times to 12%; 6 & 12, 6 times to 28%.

Loaves 1 to 6 baked by Formula I, 7 to 12 by Formula II.

7 to 12 by Formula II. The photographs show the uniformly good grain and texture obtained regardless of treatment.

Improvement from the Process of Aging

Here is a singular situation of larger loaf volumes and baking scores obtained from the two samples which had had the most severe treatment and consequent lowest grain-grading value. The shock sample threshed after the ten rains graded No. 5 sample-grade hard winter with a total damage of 16.4% (Table II), and the sample wetted six times to 28% graded No. 5 hard winter with a total damage of 15% (Table IX). In contrast to these, the sample dried in the sun and not wetted by rains and the sample wetted once to 12% both graded No. 1 dark hard winter with no damage. The loaf volumes of the samples graded with no damage were 247 cc. and 110 cc. smaller, respectively, than those which graded as damaged.

That the process of germination, if not carried too far, will increase loaf volume was shown by Willard and Swanson (1911). The loaf volumes with good textures obtained when wheat was placed under conditions of germination for 24, 48, and 72 hours were 83, 88, and 93 cubic inches respectively as compared with 85 cubic inches for the check loaf. When the germination was for longer periods, the loaf volumes were decreased and the texture was very poor. Swanson and Fenton (1933) obtained an improvement in loaf volume and texture by heating new wheat at 40°C. for as much as nine days. Heating at 45°C. produced improvement up to five days of heating, but longer periods produced deterioration. That larger loaf volumes and better texture are obtained from wheat which has had a limited amount of exposure to wetness than from new wheat which has not been so exposed seems to be a common observation.

The wheat kernel is a living organism and as such is continually undergoing change. Before flour-bleaching methods were perfected and the use of bromate introduced, flour from new wheat was much more of a problem than now. That the loaf volumes will be larger after wheat has aged was shown by Fitz (1910) who reported 2,440 cc. volume from shock-threshed wheat and milled at once, and 2,710 cc. from wheat stacked and then milled 57 days after harvest. However, if wheat is stored too long there will be a deterioration. Stockham (1920) reported that wheat stored in an elevator in North Dakota showed a significant increase in baking value during the first year. During five years thereafter there was little change in samples successively milled, but after six years there appeared a decrease in quality. Too long storage, no matter if under dry conditions, will bring deterioration. A sample of wheat

known to be about 25 years old gave a loaf volume of 1,140 cc. as compared with 2,090 for normal wheat (Swanson, 1937). The samples which gave larger loaf volumes and greater baking values, shown in Table XI, and which were graded as having 15% and 16.4% damage respectively, had attained the maximum beneficial change due to induced aging. That these two samples which gave the largest loaf volumes and the highest baking score would deteriorate in storage much more rapidly than the others which showed no damage may be inferred from existing information.

Recording Dough Mixer Curves from Representative Samples

A few curves from representative samples were made on the recording dough mixer. Unfortunately not enough flour was left to make the curves on the same flours which were used in baking, but those presented in Figure 3 show effects of representative treatments. Curves 1
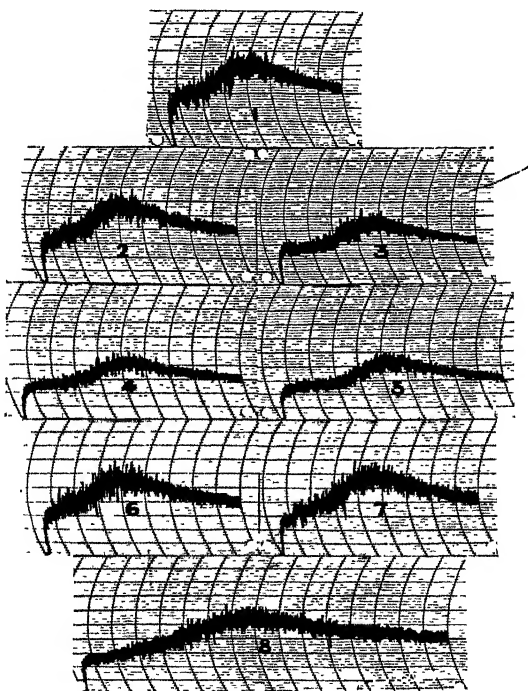
Fig. 3.   Curves from representative samples.

1. Check. Field threshed grain—no wetting.
2. Threshed from shock before rains.
3. Threshed from shock after 10 rains, 5.16 inches.
4. Threshed from outside bundles of capped shock.
5. Threshed from outside bundles of uncapped shock.
6. Grain wetted once to 26%.
7. Grain wetted three times to 24%.
8. Heat-damaged flour.

and 2 represent the pattern obtained when the wheat had suffered no damage. Curves 3, 4, and 5 show the pattern when there were increasing amounts of damage. Curve 7, from wheat wetted three times to 24%, had a notably longer development time than Curve 6, which was from wheat wetted once to 26% indicating an effect similar to that obtained when the wheat was exposed to rains. Curve 8 was made from a badly heat damaged flour and shows that one effect of damage is to increase the dough development time. Curve 3, from the average of the whole shock, does not show as much deviation from the pattern of the check curve as Curves 4 and 5, which were from wheat taken from the outside of shocks exposed to the ten rains totaling 5.16 inches. On the whole the patterns of these curves show greater differentiations due to treatments than do the baking results.

Summary

Results from exposing wheat before threshing to various amounts of moisture and also from wetting threshed wheat grain to various moisture percentages from one to six times have been presented.

Wetting wheat artificially in the straw and then drying did not produce as great physical effects as when shocked wheat was exposed to several rains. Wetting as grain produced changes somewhat similar to those caused by exposure to the rains.

The greatest apparent effects of wetting wheat were a lowering of test weight and decreases in the vitreous condition. The hardness as determined by the barley pearler was also decreased. Since these properties are important factors in grain grading, wetting wheat either artificially or by exposure to rain or by adding water to the grain and then drying did seriously affect the grading values of the grain.

The decreases in test weight were not reflected in correspondingly lower flour yields in the samples wetted after threshing. The samples wetted as grain, the test weights of which had been reduced as much as six pounds, gave essentially as high flour yields as the samples not wetted or those wetted comparatively little. This was not due to closer milling, since the ash figures varied only within the experimental limits.

Decrease in test weight is due mostly to the swelling of the kernels and partly to the roughening of the bran coat. Because there was no loss of material, there should have been no decrease in flour yield simply because the space occupied by the kernels in the test kettle had been increased. Repeated wetting may have facilitated separation of bran from the endosperm.

The baking values obtained on representative samples did not correlate with the commercial grade of the grain nor with the severity of the

treatment. An explanation for this situation is offered. The curves made on the recording dough mixer showed a longer time of development and a decrease in height as a result of the more severe treatments.

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DEVELOPMENT OF *B. MESENTERICUS* IN BREAD AND CONTROL WITH CALCIUM ACID PHOSPHATE OR CALCIUM PROPIONATE

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Many factors are known to influence "rope" development in bread. Some of these are the temperature of bread storage (Watkins, 1906), the pH of the bread (Lloyd and McCrea, 1918; Cohn *et al.*, 1918), and methods of bread baking (Amos and Kent-Jones, 1931). The possible sources of infection have received considerable attention, culminating in a tentative standard spore count of bread ingredients by Hoffman, Schweitzer, and Dalby (1937).

The technique of study of "rope" in bread has varied considerably, and our knowledge concerning several of the factors is quite general rather than reproducible by experimentation. One of the variables in experimental technique that has been handled in quite different manners by various authors has been the method of inoculation of experimental bakes. Watkins. (1906) used 1 to 20 ml of peptone wort culture. Fisher and Halton's (1928) procedure reads, "The liquor used in making the loaves was heavily inoculated with a water suspension of ropy bread." Amos and Kent-Jones (1931) used flour containing "rope" spores. Cohn *et al.* (1918) inoculated bread with 1 ml of a culture per loaf. Most of these experimenters did not determine the number of bacteria that were added to the dough.

The authors of the present paper conducted experimental bakes with determined numbers of bacteria for inoculum, hoping to eliminate one of the uncontrolled variables of previous studies. The present paper deals with investigation of the following points: (1) a study of the amount of inoculum necessary to produce a "ropy" condition in finished bread, (2) the effect of pH of the bread on development of "rope," and (3) a comparison of calcium acid phosphate and calcium propionate as inhibitors of *B. mesentericus* in bread.

Experimental Method

Cultures used: All cultures used in this study were originally obtained from "ropy" bread or from ingredients being used in bakeries experiencing "rope." These cultures were isolated through the use of the technique of Hoffman, Schweitzer, and Dalby (1937) for quantitative analysis of bread ingredients, and were purified by serial plating on

various media.   All cultures used were spore-forming rods conforming in general to the morphological description of *Bacillus mesentericus* Trevisan as given in Bergey's manual (1939).

Table I contains data on the source of the cultures and some growth characteristics.   Hoffman has used the formation of a pellicle on nutrient broth as a distinguishing character for *B. mesentericus*.   Bergey's manual, in describing this organism, states: " Broth: turbid becoming clear, with or without fragile pellicle."   As shown in Table I, culture *R* when first isolated caused turbidity but did not form a pellicle.   In later tests, culture *R* did display this ability.   Culture *Q,* on the other hand, formed a good pellicle when first isolated but lost the property of pellicle formation temporarily at a later date.   From an examination of Table I, it is evident that there is considerable variability in pellicle formation by

TABLE I

CULTURAL CHARACTERISTICS OF *B. MESENTERICUS* STRAINS USED IN STUDY

| Culture | Source | Pellicle (nutrient broth) | | | Pellicle (potato-nutrient) |
		Test 1	Test 2	Test 3	
A	Malt	?	Poor	Poor	Good
B	Bread	?	Fair	Fair	Good
G	Bread	Good	Fair	Poor	Fair
M	Rye flour	Fair	Fair	Poor	Good
N	Rye flour	Poor	Fair	Fair	Good
Q	Malt	Good	None	Fair	Fair
R	Malt	None	Poor	Poor	Fair
S	Yeast	Fair	Poor	Poor	Fair
V	Rye flour	Good	Poor	Poor	Fair
X	Bread	Good	Fair	Poor	Fair
Y	Bread	Good	Poor	?	Poor

cultures at various dates.   Perhaps nutrient broth on which these tests were conducted is not the proper medium for displaying this character. A test in which two-thirds nutrient broth was combined with one-third potato decoction showed better pellicles than on nutrient broth alone.

The cultures varied from white to red or brown in the pigment produced on potato dextrose agar.   The pink coloration formed by cultures *N* and *Y* are especially striking.   These are probably similar to the variety " ruber " described by Kent-Jones and Amos (1930).

At the beginning of the study, single strains were used in various bread bakes.   The first strains studied, *A* and *B,* were slow in developing the symptoms of " rope " in the bread.   The development of viscousness was especially slow, usually requiring 9 to 10 days at 90°F.   Strains *A* and *B* had been in culture on potato dextrose medium for about one year and may have lost their virulence.

All of the 11 cultures were then tested for virulence by inoculating sterile slices of bread and incubating these slices at 37°C. The most virulent strains were then used in combinations in later bakes. The combination G-S-X, which developed the symptoms most rapidly and consistently, was used for most of the studies.

The mixed culture G-S-X produced a " ropy " odor in bread in 1½ to 3 days, a brown discoloration in 3 to 5 days, and viscousness in 3 to 6 days when the inoculum was greater than 100,000 per loaf (368 per gram of flour). The storage temperature of the bread was 90°F (32°C) and the relative humidity 85%–88%. The effect of a smaller inoculum is described in a later portion of the paper. For most of the bakes the inoculum used was bread which had been infected with the mixed culture G-S-X. This bread, after development of " rope," was dried and powdered. The powder was thoroughly mixed and then plated to obtain a value for spores per gram of the powder. By using various amounts of dry powder, it was possible to inoculate the dough with a definite number of spores. A weighed amount of powder was suspended in 9 ml of sterile distilled water, allowed to stand for at least a half hour with intermittent, vigorous shaking. Two ml of this suspension or a dilution of it was added to 50 ml of the water for a five-loaf batch of dough. The inoculum was added to the sponge during mixing. The amount of inoculum added to any batch was double checked by platings made at the time of inoculation of the dough.

Method of baking: The bread used in this study was baked by a modified sponge and dough method, the formula for which was as follows (percentages based on weight of flour):

Sponge		%	Dough		%
Flour	815 g	60	Flour	543 g	40
Water	610 g	45	Water	350 g	25.8
Yeast	27–35 g	2.0–2.6	Salt	25 g	1.8
Yeast food	5.4 g	0.4	Sugar	79 g	5.8
			Malt	11 g	0.8
			Shortening	45 g	3.3
			Milk powder	27–108 g	2–8

The flour used was a commercial baker's brand, southeastern white flour containing 12% protein and 0.41% ash. The flour and malt used in the bakes were tested for " rope " organisms. None were found in the flour but the malt contained 10 spores per gram. No counts were conducted on the yeast which was purchased at intervals. The sponge was held 4.5 hours at 80°F (27°C) and proofing time was 30–35 minutes. The bread was baked 35 minutes at 405–410°F in a Lydon experimental oven. Each baking experiment consisted of three to four doughs producing five loaves each.

Storage of test bread and collection of data: After baking, loaves were cooled at room temperature and then heat sealed in moisture proof " cellophane," cellulose film. The bread was left unsliced. It was then stored in a cabinet with temperature controlled at 90°F (32°C) and relative humidity of 85%–88%.

The values for pH were obtained about 17 hours after baking. Measurements were made with a Beckman pH meter. The delay in taking pH may account for slight discrepancies in later tables since Fisher and Halton (1928) have shown an increase in pH of bread due to growth of *B. mesentericus.*

There were always five loaves of bread to a treatment. The first loaf was examined for " rope " symptoms at the end of 24 hours of storage. The first symptom displayed was the development of a " ropy " odor. This was followed by development of brown discoloration of the crumb, and later viscousness of the crumb. It was found that the same loaf of bread could be used for several days' examination if it were again sealed properly in " cellophane " before returning to storage.

Experimental Results

Effect of varying amounts of inoculum: Hoffman, Schweitzer, and Dalby (1937) established the following tentative standards of objectionable " rope " counts for bread ingredients : " Counts of 20 per 100 g of flour, 100 per gram of yeast or malt and 10 per gram of other ingredients." Using these counts for flour, yeast, and malt only, bread baked according to the authors' experimental formula should not contain over 814 bacteria per loaf, or about three per gram of flour. Table II gives the results of bakes containing varying amounts of inoculum per loaf and gram of flour.

TABLE II

EFFECT OF AMOUNT OF INOCULUM ON THE DEVELOPMENT OF "ROPE"

Bacteria per loaf	Bacteria per gram flour	pH of bread	Days for development of:		
			Odor	Color	Viscousness
4,000,000	14,000	5.50	1.5	3	3
380,000	1,300	5.52	2	·3	3
26,000	96	5.53	3	4	5
15,000	55	5.58	3.5	4	7[1]
1,600	6	5.58	6	7[1]	8[1]
170	0.6	5.57	10+	10+	10+

[1] In isolated spots only, not general throughout crumb.

The results of this test offer confirmation of Hoffman's criterion of safe counts of bacteria in the dough ingredients. With an inoculum of

1,600 bacteria per loaf, or 6 per gram of flour (about twice the value of Hoffman's standards for counts on flour, yeast, and malt), "rope" symptoms developed slowly under storage conditions of elevated temperature and humidity. "Rope" did not develop even after ten days of such storage when the inoculum was 170 bacteria per loaf, or about one-fifth Hoffman's criterion. As the inoculum was increased, the rate of development of symptoms also increased. Above 380,000 bacteria per loaf an increase in the inoculum did not reduce markedly the time of development of "rope" symptoms with this method of experimentation.

Effect of pH of bread on the development of "rope": Several authors (Lloyd and McCrea, 1918; Cohn *et al.,* 1918; and Morison and Collatz, 1921) have reported that the limiting hydrogen ion concentration for the growth of *B. mesentericus* lies only a few tenths of a pH below the usual pH values of commercially baked breads. Tests on the pH value of 32 brands of bread purchased in the open market show values ranging from pH 5.22 to 5.82, with an average of pH 5.50.

The recent trend toward increasing milk solids in commercially baked breads is tending to increase the pH values of the bread, and it is possible that the average pH for all breads will be increased in the future.

The present authors were interested in learning if there was variability in the rate of development of "rope" in breads varying in pH from 5.3 to 5.8. By varying the amounts of milk solids and yeast in the experimental doughs, it was possible to vary the pH of the final bread from 5.36 to 5.84.

Breads of these varying pH values were inoculated in the usual manner with "rope" bacteria. In one test the inoculum used was 260,000 bacteria per loaf (960 per gram of flour). In the other test one-twenty-seventh of this number or 9,600 bacteria per loaf were used (35 per gram of flour). Table III gives the results of these two tests. When the high inoculum was used, the difference in the rate of development of "rope" symptoms at the three pH levels was very small. The only symptom which varied to any extent was the development of viscousness. At pH 5.8 viscousness developed in three days, while at pH values below this it required four days.

With only 9,600 bacteria per loaf, a greater difference becomes apparent at the lower pH value. A half day longer was required for the development of odor, two days for color, and three days longer for viscousness at pH 5.37 than was required at pH 5.57. Above pH 5.57 there was little difference except that a half day less was required for odor development at pH 5.87. The results of these two tests show only slight differences in development of symptoms from the results given in Table II for comparable inoculum and pH of bread.

TABLE III

EFFECT OF BREAD pH ON DEVELOPMENT OF "ROPE"

Original pH of bread	Milk solids used	Yeast used	Bacteria per loaf	Days for development of:		
				Odor	Color	Viscousness
	%	%				
5.84	8.0	2.0	260,000	2.5	2.5	3
5.70	6.0	2.2	260,000	2.5	2.5	4
5.36	2.5	2.6	260,000	2.5	2.5	4
5.87	8.0	2.0	9,600	3.5	5.0	6
5.69	5.0	2.2	9,600	4.0	5.0	6
5.57	4.0	2.6	9,600	4.0	5.0	6
5.37	2.0	2.6	9,600	4.5	7.0[1]	9[1]

[1] In isolated spots only, not general throughout crumb.

Inhibitory effect of calcium acid phosphate: Calcium acid phosphate has been used for a number of years in commercial bread baking as a means of controlling the development of *B. mesentericus.* The usual recommendation for the amount of this material used is 0.20%–0.25%. (If symptoms of " rope " develop, double these amounts are recommended.) Fisher and Halton (1928) compared the effect of several acidic ingredients on the development of " rope " and found that calcium acid phosphate possessed greater inhibitory action than did phosphoric acid itself. They attributed this surprising result to the better distribution of the calcium salt than of the acid during mixing of the dough, with a correspondingly more even distribution of pH throughout the crumb of the bread produced. With an equivalent lowering of pH, calcium acid phosphate was found more effective than tartaric acid, but no satisfactory explanation of this fact was made. Fisher and Halton, nevertheless, came to the general conclusion that the inhibitory action of calcium acid phosphate is mainly due to lowering the pH.

The present studies included the effect of calcium acid phosphate on the final pH of the baked bread. Bakes were made using the same concentration of phosphate in breads of different pH values. As previously mentioned, the different pH values were obtained by varying the amounts of milk solids and yeast.

With the flour used, the effect of any one concentration of calcium acid phosphate in lowering the pH was about the same, irrespective of the pH of the untreated bread. Thus 0.25% of calcium acid phosphate lowered the pH about 0.14, whether the untreated bread had a pH of 5.85 or 5.44. Using 0.5% of calcium acid phosphate, the pH was lowered in the range of 0.34. Fisher and Halton have shown that a final pH of phosphate-treated bread of about 5.2 is required for inhibition of the " rope " organism. Thus the pH of the bread without phosphate

would have to lie in the range of 5.35 if appreciable inhibition were to be obtained through the use of 0.25% of calcium acid phosphate.

In order to obtain a proper evaluation of the effect of calcium acid phosphate as an inhibitor, it was believed that tests in which the amount of inoculum was varied would be necessary for a basis of comparison. Accordingly, 0.25% of calcium acid phosphate was used in a test bake in which varying amounts of bacteria were added to the sponge.

Table IV gives the results of this test, indicating " days for appearance " of the various symptoms and the " days of inhibition." The latter were computed by subtracting the " days for appearance " of Table II from Table IV at approximately equal levels of inoculum.

TABLE IV

"Rope" Inhibition Obtained with 0.25% Calcium Acid Phosphate in Bread Containing Varying Amounts of Inoculum

Bacteria per loaf	pH of bread	Days for appearance of:			Days of inhibition of:		
		Odor	Color	Viscousness	Odor	Color	Viscousness
4,900,000	5.31	5	6	8	3.0	3	5
490,000	5.34	6	7	9	3.5	4	6
37,000	5.36	8	10	10	5.0	6	5

Considering the time required for the appearance of various symptoms, it is evident that the amount of inoculum greatly influences the effect of calcium acid phosphate in retarding " rope " development. The more bacteria, the less effective is the phosphate.

It is also apparent from Table IV that 0.25% phosphate is effective in bread with a final pH above 5.2, if the inoculum is relatively low. The inhibition produced was better than that obtained by Fisher and Halton, who undoubtedly used higher inoculum.

To further test the effect of calcium acid phosphate as a " rope " inhibitor, a series of bakes were made using varying amounts of phosphate in breads with different pH values and varying inoculum. The amount of inoculum in all cases was relatively high. In most bakes, controls with inoculum but without phosphate were tested. The variations in symptom development for controls were as follows: odor 1.5–3 days, color 3–5 days, viscousness 3–6 days.

The results of several tests are compiled in Table V. Conclusions which may be drawn from this table are:

1. Calcium acid phosphate at 0.5% produces effective inhibition of " rope " in bread the pH of which would be 5.84 without the phosphate and with the phosphate 5.40. Other investigators, who state that the

pH value of the phosphate-treated bread must be 5.20 or lower for effective inhibition, probably used very high numbers of bacteria.

2. A phosphate concentration of 0.375% is an effective inhibitor in breads of final pH value of 5.4 or lower.

3. A concentration of 0.25% phosphate has little retarding effect in breads of high pH, but as the pH of the bread is lowered more effect is observed.

TABLE V

EFFECT OF VARYING AMOUNTS OF CALCIUM ACID PHOSPHATE ON "ROPE" DEVELOPMENT

Amount of phosphate	pH of untreated bread	pH of treated bread	Bacteria per loaf	Days for appearance of:		
				Odor	Color	Viscousness
%						
0.25	5.80	5.70	360,000	3	4	4
0.25	5.60	5.50	360,000	5	7	9+
0.25	5.50	5.36	490,000	6	7	9
0.25	5.45	5.25	2,360,000	6	6	11
0.375	5.59	5.40	340,000	8	9	11+
0.375	5.43	5.18	1,640,000	8	11+	11+
0.5	5.84	5.40	340,000	9	9	10+
0.5	5.59	5.21	340,000	11+	11+	11+

Inhibitory effect of calcium propionate: The salts of propionic acid, especially calcium and sodium propionate, have been used for several years as mold inhibitors for bread and bakery products (Pyler, 1938). Weimershaus and Svenson (1938), using propionic acid rather than calcium propionate, found 0.25% to completely prevent "rope." The acid-treated bread required longer fermentation time and the volume of the bread was less than the untreated. The investigators gave no details on experimental methods. Commercial baking experience with calcium propionate has shown this material to be an effective "rope" inhibitor, but concentrations which are effective for inhibition under various conditions have not been reported in the literature.

The inhibitory action of propionates does not depend on lowering the pH of the bread as in the case of the calcium acid phosphate. Hoffman, Schweitzer, and Dalby (1939) have shown that the acids of the acetic series up to lauric are inhibitory to fungi when used in a medium with a pH value below 7.

Breads baked with varying amounts of propionate salts did not show a consistent change in pH from that of the control bread of the same bake. It was usually found that the pH value of the propionate-treated bread was a few hundredths lower than that of the control bread. Often,

however, the reverse was true. Of 32 bakes, only three showed greater variation in pH than 0.05 from the controls. No correlation was found between the amount of propionate salt added and the resulting pH variation from the control bread. Unlike calcium acid phosphate, the propionates exhibited inhibitory action without markedly changing the pH of the bread.

The effect of varying amounts of inoculum on inhibition of " rope " in bread containing 0.11% of calcium propionate was studied in a manner similar to that used in the study of calcium acid phosphate. Table VI

TABLE VI

"Rope" Inhibition Obtained with 0.11% Calcium Propionate in Bread Containing Varying Amounts of Inoculum

Bacteria per loaf	pH of bread	Days for appearance of:			Days of Inhibition of:		
		Odor	Color	Viscousness	Odor	Color	Viscousness
9,700,000	5.54	5	7	8	3.5	4	5
980,000	5.53	8	11	13	6	8	10
72,000	5.54	12	13	13	9	9+	8+

gives the results of this study. Unfortunately the inoculum for this test was about double that given in the study of calcium acid phosphate in Table IV. However, by interpreting Tables IV and VI, and considering results obtained in Table II, it is possible to draw a fair correlation.

It is first of all evident that 0.11% of propionates is more effective than 0.25% of calcium acid phosphate at all levels of inoculum in bread of approximately similar original pH. Propionates apparently delay the development of color and viscousness more than does calcium acid phosphate. Thus in Table IV there was a spread of only two to three days between the time of odor and viscousness development with calcium acid phosphate, while this spread was three to five days with calcium propionate.

Table VII gives the results of bakes with various amounts of calcium propionate at different pH levels. The authors believe the following conclusions can be drawn from Table VII:

1. Calcium propionate at 0.188% produces effective inhibition of " rope " in bread the pH of which is as high as 5.8. At pH 5.6 a concentration of 0.156% will produce as good inhibition.

2. At 0.11% calcium propionate produces considerable inhibition even when the pH of the bread is quite high and the retarding effect becomes more marked as the pH is lowered.

3. Lower concentrations of calcium propionate are also somewhat effective if the pH of the bread is reduced. Thus as little as 0.075% calcium propionate shows some retarding action in bread of pH 5.4.

TABLE VII

EFFECT OF VARYING AMOUNTS OF CALCIUM PROPIONATE ON "ROPE" DEVELOPMENT

Amount of propionate	pH of untreated bread	pH of treated bread	Bacteria per loaf	Days for appearance of:		
				Odor	Color	Viscousness
%						
0.075	5.40	5.40	320,000	5	6	7
0.11	5.80	5.69	15,000,000	6	8	9
0.11	5.66	5.66	1,220,000	10	10	11
0.11	5.55	5.54	980,000	8	11	13
0.156	5.60	5.60	560,000	12	12+	12+
0.156	5.45	5.43	2,400,000	10	11+	11+
0.188	5.84	5.83	340,000	9	9	10+
0.188	5.50	5.52	440,000	12+	12+	12+

Discussion

A great hindrance to the advancement of our knowledge of the development of "rope" in bread is the lack of opportunity to study the trouble under bakery conditions. No baker wants tests conducted in his plant with these bacteria, and when an outbreak of rope occurs every effort is directed toward elimination of the conditions producing the disease or toward corrective measures.

One of the vexing questions in connection with the trouble is the part played by the inoculum from ingredients and the part played by inoculum from contaminated equipment. Ashby and Prickett (1938) have pointed out the danger of contaminated piping connecting tank scale and mixer. Such contamination undoubtedly accounts for sporadic outbreaks of "rope." What the amount of inoculum under these conditions may be is unknown, but judging from the rapid deterioration of the bread in many of these epidemics it must be very high.

Hoffman, Schweitzer, and Dalby (1937) have given some data on maximum spore counts of bread ingredients studied over a period of several years. The maximum for flour was 150 spores per gram, for yeast 20,000 per gram, for malt 10,000 per gram, and as high as 100,000 in other malted materials. Using these maxima for flour, yeast, and malt, it would be possible to introduce an inoculum of 200,000 spores per loaf according to the formula used in the present study. This is less than the inoculum used in many tests reported in this paper, and is probably also less than that used by investigators who inoculated by means of "ropy" bread or spore suspensions.

Amos and Kent-Jones (1931) reported "ropy" bread produced with less than ten spores per gram of flour while breads baked with other

flour containing 160 spores per gram failed to become "ropy." From these results the authors concluded that baking practices were of more importance than the inoculum of the ingredients. We feel that our studies show the amount of inoculum to be of greater importance than supposed by Amos and Kent-Jones. With high inocula, the effect of changing baking practice is greatly reduced as is shown in Table III. Also the effect of corrective measures is reduced as is shown in Tables IV and VI.

Summary

The development of "rope" in bread was studied by means of laboratory bakes in which the number of bacteria used for inoculum was a controlled factor.

"Rope" symptoms developed with increasing rapidity as the inoculum was raised from 6 to 1,300 bacteria per gram of flour. Larger inoculum than 1,300 bacteria per gram did not materially increase the rate of development of "rope."

Hoffman and his associates proposed standards for bacterial counts of bread ingredients. Bread baked with six bacteria per gram of flour (twice Hoffman's standard adjusted to our formula) developed symptoms very slowly. At one-third Hoffman's standard, no "rope" developed in ten days.

The pH of bread did not influence the rate of "rope" development if the inoculum used was high. With lower inoculum, the rate of development varied markedly when pH 5.37 was compared with pH 5.87.

The inhibitory effects of both calcium acid phosphate and calcium propionate were found to be influenced by the amount of inoculum used and by the pH of the bread.

Calcium propionate was found to be two to three times as effective as calcium acid phosphate. The difference in retarding effect between the two "rope" inhibitors was greatest at high pH value.

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THE STARCH DEGRADING PROPERTIES OF
BARLEY MALTS [1]

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The ability of cereal amylases to hydrolyze starch and dextrins is largely responsible for their extensive use in industry. Many amylase studies have been carried out with regard to the utilization of malts in the brewing and distilling industries, and in flour technology. In addition, malt amylase finds applications in the preparation of special dextrins and sugars, in the determination of starch, in the removal of undesirable starch and dextrins from materials such as sorgo juice (Walton, Ventre, and Byall, 1940), and elsewhere. It is obvious that the most advantageous use of malt in such diverse fields must be accompanied by a complete understanding of the nature and amount of amylase in the malt.

It is generally accepted that malt amylase has three important functions in starch hydrolysis, namely saccharification, dextrinization, and liquefaction. Two components, alpha- and beta-amylase, acting independently or in combination, are recognized as performing these functions. Present knowledge indicates that alpha-amylase is essentially a liquefying and dextrinizing amylase, while the beta component is the

major factor promoting saccharification. However, with unmodified malt extract, both saccharification and dextrinization are the result of the combined actions of the two components. On the other hand starch liquefaction has been proposed as a measure of alpha-amylase even in the presence of beta-amylase (Blom, Bak, and Braae, 1937; Józsa and Johnston, 1935; Hollenbeck, 1939). Space does not permit a complete review of the literature dealing with the properties and actions of the malt amylases. The reader is referred to reviews such as those by Hanes (1937), Hesse (1934), Lüers (1936), van Klinkenberg (1934), and Blish, Sandstedt, and Kneen (1938), and to two recent papers by Sandstedt, Kneen, and Blish (1939), and Kneen and Sandstedt (1941).

A malt well adapted to the performance of a specific function has frequently been found deficient when applied under other circumstances. Lack of methods for absolute differentiation between the amylase components has hindered any adequate estimation of the various amylolytic potentialities of malt. Methods proposed by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) have made practical the accurate determination of alpha- and beta-amylase activities [3] in the presence of each other. Using these and other methods a study has been made of the starch-degrading properties of twelve barley malts.[4] .

Methods

"Free" extract: The malt grain was ground finely (83% passing through a 1-mm sieve, 42% through a 0.5-mm sieve) and 1 g of meal extracted with 100 ml of water for 1 hour at 30°C. After 5 minutes of centrifuging the extract was poured rapidly through cotton and preserved with toluol. The only exception to this procedure was in the preparation of the extract used for raw starch hydrolysis; here extracts 5 times as strong were used.

In connection with the preparation of the malt extract the question arose as to the degree of standardization necessary in the grinding procedure and in the ratio of meal to extraction liquid. Sandstedt, Kneen, and Blish (1939) reported that there was considerable latitude allowable in the grinding of malts previous to securing an extract for alpha-amylase determination. For the present study a barley malt was ground to 5 different degrees of fineness by a burr mill. To indicate variation in fineness the amount passing through a 0.5-mm sieve was determined for each of the meals. Progressing from finest to coarsest grind these amounts were respectively 42%, 36%, 32%, 27%, and 23%. Extrac-

tions were made and saccharogenic units (Kneen and Sandstedt, 1941) determined. The values found were respectively 19.5, 19.4, 19.7, 19.2, and 19.6 units. This variation is well within the limits of experimental error. To investigate the effect of varying the proportion of malt to extraction liquid 1, 2, 3, and 4 g of a low diastatic malt were extracted in the usual manner with 100 ml of water. Saccharogenic activities were determined and unit values of 8.0, 8.0, 7.9, and 8.0 were found. This deviation likewise is insignificant. Obviously then, when determining amylase activity, considerable latitude is permissible in the preparation of malts for extraction and in the ratio of meal to extraction liquid.

" *Total* " *extract:* To 4 g of the finely ground meal 0.4 g Merck's papain was added and extraction made with 100 ml of dilute calcium acetate solution (1 g calcium acetate per liter) for 18 hours at 30°C. Toluol was used as a preservative. After centrifuging and filtering the extract was diluted 1 to 4 with water before use. The papain had adequate activity in this concentration. Increasing the ratio of papain to meal or treatment with papain " activators " gave no increase in amylase extraction.

Malt saccharogenic activity (method of Kneen and Sandstedt, 1941): Using 20 ml of buffered 2% Lintner starch, plus extract and water sufficient to give a total volume of 30 ml, the degree of saccharification taking place in 15 minutes was determined and *malt saccharogenic units* (the number of grams of soluble starch converted to maltose by 1 gram of malt in 1 hour at 30°C) were calculated. Such precautions as preparing the starch and calculating the units on a dry weight basis, and subtracting a starch-extract " blank " were observed. A convenient method for determining the starch-extract blank is to add to the starch and water in the reaction flask the 20 ml of 1% sulfuric acid which the method normally employs to stop enzyme action, then add the extract and determine the ferricyanide reduction brought about by 5 ml of the mixture. The further precaution of using malt equivalents such that 40% conversion of starch to maltose is not exceeded was satisfied for malts up to about 240° Lintner by using for hydrolysis 2 ml of a 1-to-100 " free " extract or 1 ml of a 1-to-100 " total " extract.

Alpha dextrinogenic (alpha-amylase) activity: Alpha-amylase activity was determined by the method of Sandstedt, Kneen, and Blish (1939). In this method the rate of dextrinization in the presence of an excess of beta-amylase is measured and activity expressed in terms of *alpha-amylase units* (the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by 1 gram of malt in 1 hour at 30°C).

Beta saccharogenic (beta-amylase) activity: From the malt saccharogenic and alpha dextrinogenic activities the beta-amylase activity

was calculated according to the technique of Kneen and Sandstedt (1941). In this technique the maltose production attributable to alpha-amylase alone is determined and subtracted from the maltose produced by the action of unmodified malt extract on soluble starch. The difference represents maltose production by the action of the beta component. Results are recorded as *beta-amylase units* (the number of grams of soluble starch converted to maltose by the beta-amylase of 1 gram of malt in 1 hour at 30°C).

Malt dextrinogenic activity: As used in this communication the term "malt dextrinogenic activity" signifies the ability of unmodified malt extract to dextrinize soluble starch. It was determined under conditions identical to those used for alpha dextrinogenic activity with the exception that the addition of an excess of beta-amylase was omitted. Accordingly, to 20 ml of 2% buffered Lintner starch were added water and extract sufficient to bring the total volume to 30 ml and dextrinization carried out at 30°C. Dextrinization time was recorded in minutes as the period elapsing between the start and the point at which the reaction mixture gave with iodine the "red-brown" color described by Sandstedt, Kneen, and Blish (1939). *Malt dextrinogenic units* were calculated as the number of grams of soluble starch dextrinized by 1 gram of malt in 1 hour at 30°C.

Starch-liquefying activity: Liquefying activity was determined by the method of Józsa and Gore (1930) as modified by Hollenbeck (1939). The modifications consisted of using glass beakers for starch pasting containers, a slightly different starch concentration (4.36 g dry starch per 100 g suspension), and a reaction temperature of 30°C. Liquefaction was measured over a 30-minute period. Percent decline in outflow time was calculated from the outflow times of unmodified starch, starch after 30 minutes of malt liquefaction, and completely liquefied starch. The actual weight of starch liquefied in 30 minutes was calculated from a pre-established relationship between decline in outflow time and grams of starch liquefied. The data were translated to conform with those of Józsa and Johnston (1935) and "liquefons" calculated from their published tables. *Starch-liquefying activity* was then expressed as "liquefons per gram of malt." The values as calculated represent a 1-hour run at 30°C and therefore are considerably higher than would be found at the 21°C reaction temperature used by Józsa and Johnston.

Activity on raw starch: The action of the malt extracts on raw (unboiled) starch was measured in a manner similar to the technique used by Blish, Sandstedt, and Mecham (1937). To 10 g of raw wheat starch were added 10 ml of a 1-to-20 malt extract and sufficient water and acetate buffer [5] (pH 4.7) to bring the total liquid volume to 100 ml. Toluol

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[5] Buffer concentration was 3.0 ml glacial acetic acid and 4.1 g anhydrous sodium acetate per liter of reaction liquid.

was used as a preservative and hydrolysis carried out at 30°C. Aliquots for the determination of reducing substances by the ferricyanide method were taken after 4 and 24 hours of action. Reducing power was arbitrarily calculated as milligrams of maltose and the *activity on raw starch* expressed as milligrams of maltose produced by 1 g of malt in the 20-hour interval between the 4th and 24th hours of hydrolysis.

Autolytic diastatic activity: The method of Blish and Sandstedt (1933) was used to determine maltose production by the autolysis of the barley malt meals. To 5 g of meal were added 46 ml of acetate buffer (pH 4.7) and the mixture digested for one hour at 30°C. The reaction was stopped with sulfuric acid and sodium tungstate and reducing substances determined in the filtrate by the ferricyanide method. Using the method of Sandstedt (1939) for reducing sugars in flour, blank determinations were made on all the samples and these values subtracted from those obtained by autolysis. This resulted in an estimation of the actual amount of reducing substances produced. *Autolytic diastatic* activity was calculated as milligrams of maltose produced by the autolysis of 1 gram of malt for 1 hour at 30°C.

Results

All the data obtained on the twelve malts are presented in Table I. In this table the malts are arranged in the order of their Lintner values.[6]

TABLE I
STARCH-DEGRADING PROPERTIES OF "FREE" AND "TOTAL" BARLEY MALT EXTRACTS AND AUTOLYTIC DIASTATIC ACTIVITIES OF THE MEALS

Malt	Free extract							Autolytic diastatic activity	Total extract			
	Lintner value	Malt saccharogenic activity	Beta-amylase activity	Malt dextrinogenic activity	Alpha-amylase activity	Starch-liquefying activity	Activity on raw starch		Malt saccharogenic activity	Beta-amylase activity	Malt dextrinogenic activity	Alpha-amylase activity
	deg	units	units	units	units	liquefons per g	mg maltose	mg maltose	units	units	units	units
A	247	33.7	30.5	38.9	64.2	1227	1690	113	43.1	38.4	50.1	95.6
B	215	30.6	23.4	51.7	145.5	2876	2010	155	40.6	32.0	60.9	172.7
C	208	30.3	25.3	42.3	101.3	2060	1930	132	39.4	32.9	55.8	132.6
D	187	23.9	22.4	22.8	30.8	602	1360	82	35.7	33.5	30.0	44.0
E	168	23.6	20.2	34.6	66.1	1227	1760	126	27.4	22.8	40.6	92.7
F	154	20.7	18.5	26.7	43.5	787	1250	98	26.4	23.6	33.0	57.7
G	147	18.9	16.7	23.4	45.1	882	1280	94	25.9	22.9	33.2	61.4
H	133	18.0	14.5	28.8	69.4	1255	1800	121	22.3	17.6	35.6	92.3
I	116	15.1	12.3	22.7	55.8	1066	1480	97	18.6	15.1	28.7	69.6
J	113	14.8	13.0	18.0	36.9	655	1220	75	19.6	17.3	24.2	45.1
K	88	11.9	8.8	19.9	61.1	1227	1540	103	14.8	11.0	25.2	71.9
L	71	8.8	6.8	13.5	38.8	697	1200	76	15.9	13.4	22.9	49.6

[6] Lintner values were supplied by Dr. Allan D. Dickson of the Wisconsin Station.

Saccharogenic, dextrinogenic, beta-amylase, and alpha-amylase activities are given for both the " free " and " total " (papain) extracts. In addition starch-liquefying activity and activity on raw wheat starch are given for the free extracts and autolytic diastatic activities for the malt meals.

In order to minimize the possibility of enzymic changes during aging of the dry malts the data recorded in Table I were all obtained as rapidly as possible following the determination of the Lintner values. Much of the data was checked by two and in some cases three investigators, and it is regarded as possessing a high degree of accuracy. In view of the complexity of Table I various interrelations between the amylase activities will be discussed separately.

Discussion

Saccharogenic activity: Table II lists relative data comparing Lintner values and free saccharogenic and beta-amylase activities for the twelve malts. The data in each column were calculated on the basis of assign-

TABLE II

RELATIONSHIP BETWEEN LINTNER VALUES, MALT SACCHAROGENIC UNITS
(KNEEN AND SANDSTEDT, 1941) AND THE ACTIVITY OF
THE BETA-AMYLASE COMPONENT

| Malt | Free extract | | |
	Lintner value	Malt saccharogenic activity	Beta-amylase activity
A	100	100	100
B	87	91	77
C	84	90	83
D	76	71	73
E	68	70	66
F	62	61.	61
G	60	56	55
H	54	53	48
I	47	45	40
J	46	44	43
K	36	35	29
L	29	26	22

ing a value of 100 to sample A. It is obvious that there is a high degree of correlation between saccharogenic activity as determined by the method of Kneen and Sandstedt (1941) and as determined by the conventional " Lintner " method. While agreement is not absolute it is probably as close as might be expected when comparing data obtained by two different methods in two separate laboratories. Numerically the Lintner values average 7.5 times the saccharogenic units. Many cereal laboratories are not equipped for determining saccharogenic activity by the Lintner technique but are equipped for the determination of " Kneen-

Sandstedt units." Pending the accumulation of more data it appears that this factor of 7.5 may be used for converting saccharogenic units to degrees Lintner or *vice versa*.

The data of Table II show that there is good correlation between beta-amylase activity and malt-saccharogenic activity. This indicates that beta-amylase is the component principally responsible for malt saccharification. Actually the degree of saccharification due to beta-amylase alone ranges from 74.0% for malt K to 93.7% for malt D, with an average value of 84.1% for the twelve malts under consideration. These figures agree fairly well with the data of Hills and Bailey (1938), who estimated that from 25% to 29% of the saccharifying action of germinated barley was due to alpha-amylase. However, it should be emphasized that, in the comparison of two malts, higher saccharogenic activity does not necessarily mean higher beta-amylase content. For example the relatively high *alpha-amylase* contents of malts B and I result in their exhibiting a higher saccharogenic activity than would be predicted solely on the basis of their beta-amylase contents.

TABLE III

ALPHA-AMYLASE ACTIVITY AND ITS RELATION TO STARCH LIQUEFACTION, RAW STARCH DEGRADATION, AND AUTOLYTIC DIASTATIC ACTIVITY

Malt	Free extract				Autolytic diastatic activity
	Alpha dextrinogenic activity	Starch liquefying activity	Activity on raw starch	Malt saccharogenic activity	
B	100	100	100	100	100
C	70	72	96	99	85
H	48	44	90	59	78
E	45	43	88	77	81
A	44	43	84	110	73
K	42	43	77	39	66
I	38	37	74	49	63
G	31	31	64	62	61
F	30	27	62	68	63
L	27	24	60	29	49
J	25	23	61	48	48
D	21	21	68	78	53

Alpha-amylase activity: In Table III are listed relative data for alpha-amylase activities of the free extracts and for those actions which appear to be related to the alpha-amylase content of the malts. These include starch liquefaction, activity on raw starch, and autolytic diastatic activity. In addition values for malt saccharogenic activity are included for comparison. The data in each column were calculated on the basis of assigning a value of 100 to sample B and the samples listed in order of decreasing alpha-amylase activity.

From Table III it is apparent that there is excellent agreement between alpha dextrinogenic activity and the capacity of a malt to liquefy starch. It may be concluded that, in the ratio of malt to starch used, the beta-amylase content of the extract had no effect on the rate of liquefaction. Likewise the data indicate that the alpha-amylase activity of a malt extract may be measured equally well by both the dextrinization method of Sandstedt, Kneen, and Blish (1939) and the liquefaction technique of Józsa and Johnston (1935). Numerically the values for "liquefons per gram" average 19.0 times those for "alpha-amylase units," and it appears that this factor may be used for interconversion between the two types of alpha-amylase values when both are determined at 30°C. However, the liquefying and dextrinizing actions of alpha-amylase should not be confused. Liquefaction by alpha-amylase is characterized by inappreciable production of dextrins and may be just a preliminary step before the eventual dextrinization of the starch takes place (Hollenbeck and Blish, 1941). In addition, the "amylophosphatase" conception of Waldschmidt-Leitz, Samec, and Mayer cannot be ignored. As summarized by Mayer (1939) the findings of these authors indicate that enzymic starch liquefaction can take place without the production of either dextrins or reducing sugars. The present status of the problem suggests that starch liquefaction as measured by the method of Józsa and Johnston (1935) be designated as "alpha liquefying activity" and starch dextrinization in the presence of an excess of beta-amylase (Sandstedt, Kneen, and Blish, 1939) as "alpha dextrinogenic activity."

The data of Table III show that the ability of malt to hydrolyze raw wheat starch is highly correlated with alpha-amylase activity. The small spread between the actions of those malts of highest alpha-amylase activity resulted in large part from the ratio of malt extract to raw starch. When half concentrations of malts B and C were used on the regular amount of starch the relative values for these two samples were respectively 100 and 88. The raw-starch activity of sample B shows lack of correlation with alpha-amylase activity. Upon further investigation this extract was found to be abnormally high in maltase activity. The amount of glucose produced from preformed maltose during a 24-hour hydrolysis may be appreciable. As pointed out by Blish, Sandstedt, and Kneen (1938) when all ferricyanide reducing substances are calculated as maltose, as in the measurement of activity on raw starch, the *apparent* maltose production is higher than actually occurs and malt activity is incorrectly evaluated.

The close relationship between alpha-amylase activity and activity on raw starch also suggests the strong possibility that the "raw starch factor" of Blish, Sandstedt, and Mecham (1937) may be alpha-amylase.

"Alpha-amylase values" as reported by these authors and by Blish, Sandstedt, and Kneen (1938) were determined by the unmodified Wohlgemuth technique (dextrinization by the combined action of alpha- and beta-amylase) and as such do not represent a measure of the actual alpha-amylase activity. Conclusions which these authors based on comparisons between "alpha-amylase activity" and activity on raw starch are therefore of questionable value. The present data support the hypothesis of Brown and Morris (1890), who concluded that the amylase responsible for disintegrating raw starch was the "liquefying" enzyme which appears when grain is germinated.

The autolytic diastatic activity of malt was found by Sallans and Anderson (1939) to be correlated with liquefying activity of the extract. The work of these authors also confirmed the finding of Shellenberger and Bailey (1936) that autolytic activity was not correlated with the saccharifying power of the malt extract. The results shown in Table III likewise indicate a fairly good correlation of autolytic diastatic activity with alpha-amylase content and a lack of correlation with malt saccharogenic activity. The data therefore confirm the conclusion that alpha-amylase content is a major factor governing the rate of autolytic maltose production by malt meals. Sallans and Anderson (1939) also found a significant correlation between malt-saccharifying and malt-liquefying activities. The data of Table III are not in agreement [7] with this finding and indicate that the alpha-amylase activity of a malt can *not* be predicted on the basis of the saccharogenic activity of the extract.

The fact that saccharogenic activity is of little value in estimating the alpha-amylase activity of malt assumes considerable importance in the cereal industry. When malt is used as a flour supplement it is being introduced into a medium already well supplied with beta-amylase. Under these conditions the alpha component of malt amylase must be considered as having major significance. Maximum influence on the flour would be expected from malts of high alpha-amylase content, not necessarily from those of high saccharogenic power.

Malt dextrinogenic activity: That beta- and alpha-amylase are both operative in the rate at which the action of malt causes starch to lose its ability to give the typical starch-iodine color reaction has been recognized by Blom, Bak, and Braae (1937) and Hanes and Cattle (1938). Sandstedt, Kneen, and Blish (1939) found that when an excess of beta-amylase was present, dextrinization of soluble starch proceeded nearly six times as fast as when the same amount of alpha-amylase acted alone. Further studies indicate that this ratio is slightly too high. The average value for numerous determinations was found to be 5.4; that is, in the

[7] The correlation coefficient between malt-saccharifying and malt-liquefying activities was found to be + .6026.

presence of an excess of beta-amylase the rate of dextrinization of soluble starch is 5.4 times as rapid as would be caused by the equivalent amount of alpha-amylase acting independently on such starch. A "true" measure of alpha-dextrinogenic activity would therefore be attained by dividing the alpha-amylase "units" of Sandstedt, Kneen, and Blish (1939) by 5.4.

Table IV shows the manner in which the alpha- and beta-amylase activities of the twelve malts are related to the dextrinogenic activities. Data are listed for beta-amylase activity (A), alpha-amylase activity divided by 5.4 (B), the summation of these two activities (A + B), and for malt dextrinogenic activity. With the possible exception of sample D there is rough numerical agreement between the unit values determined for malt-dextrinogenic activity and the values derived from the summa-

TABLE IV

THE RELATIONSHIP OF ALPHA- AND BETA-AMYLASE CONTENTS OF
MALT TO STARCH DEXTRINIZATION

Malt	A Beta-amylase activity	B Alpha-amylase activity	A + B	Malt dextrinogenic activity
	units	*units/5.4*		*units*
A	30.5	11.9	42.4	38.9
B	23.4	27.0	50.4	51.7
C	25.3	18.8	44.1	42.3
D	22.4	5.7	28.1	22.8
E	20.2	12.2	32.4	34.6
F	18.5	8.1	26.6	26.7
G	16.7	8.4	25.1	23.4
H	14.5	12.9	27.4	28.8
I	12.3	10.3	22.6	22.7
J	13.0	6.8	19.8	18.0
K	8.8	1r.3	20.1	19.9
L	6.8	7.2	14.0	13.5

tion of the beta-saccharogenic and "true" alpha-dextrinogenic units. Further investigation must be carried out before any final conclusion may be drawn regarding this possible additive nature of alpha- and beta-amylase in dextrinization. However, it does appear that an approximate evaluation of the *beta*-saccharogenic activity of a malt may be obtained simply by subtracting alpha-amylase units (divided by 5.4) from malt-dextrinogenic units.

Since the action of beta-amylase alone does not appreciably alter the color which starch gives with iodine, it would seem that the chief function of this component in dextrinization is to degrade the starch to alpha-amylodextrin. A further function is indicated by the fact that the presence of beta-amylase likewise increases the rate at which alpha-amylodextrin is dextrinized by alpha-amylase (Sandstedt, Kneen, and Blish,

1939).   The role of beta-amylase in dextrinization then becomes the degradation of starch to alpha-amylodextrin followed by the hydrolysis of some of the products resulting from the action of alpha-amylase on this dextrin.

"*Latent*" *amylase:* The latent amylase of germinated or ungerminated barley may be designated as that fraction which requires the action of a proteolytic enzyme such as papain to render it extractable by water. In Table V data are listed showing the amounts of latent amylase and the percentages of total amylase in the latent condition for both the alpha- and beta-components.

The data of Table V show that, as with the free amylases, there is wide variation in the amounts of latent beta- and alpha-amylase in malts. With the exception of one or two individual samples a fairly constant percentage of the total amylase exists in the latent state.   The amounts

TABLE V
The Latent Alpha- and Beta-Amylase of Malts

Malt	Beta-amylase activity		Alpha-amylase activity	
	Latent	Relation of latent to total	Latent	Relation of latent to total
	units	%	*units*	%
A	7.9	20.6	31.4	32.9
B	8.6	26.9	27.2	15.8
C	7.6	23.1	31.3	23.6·
D	11.1	33.1	13.2	30.0
E	2.6 .	11.4	26.6	28.7
F	5.1	21.6	14.2	24.6
G	6.2	27.1	16.3	26.6
H	3.1	17.6	22.9	24.8
I	2.8	18.5	13.8	19.8
J	4.3	24.9	8.2	18.2
K	2.2	20.0	10.8	15.0
L	6.6	49.3	10.8	21.8
Average		24.5		23.5

varied from 11.4% to 49.3% for the beta component and from 15.0% to 32.9% for the alpha component.   The average percentages of latent amylase were about the same for the two components: 24.5% of the beta-amylase and 23.5% of the alpha-amylase.   This agreement does not hold for individual malts.   For example 49.3% of the beta-amylase was latent in sample L and only 21.8% of the alpha component.

The necessity for including determinations of "total" amylase in malt-analysis programs has not been established.   Certainly in instances where prolonged action of malt on starch substrate is permitted some attention should be paid to the amylase liberated by proteolytic activity.

Summary and Conclusions

Values for malt saccharogenic activity, beta-amylase activity, malt dextrinogenic activity, and alpha-amylase activity were determined for both the " free " and " total " (papain) extracts from twelve barley malts. In addition starch-liquefying activity and " activity on raw starch " were determined for the " free " extracts and autolytic diastatic activity for the malt meals.

The data presented indicate that no one of the methods commonly used for determining the starch-degrading property of malt can be expected to provide a complete picture of the amylolytic potentialities. Individual determinations of saccharification, dextrinization, or liquefaction of starch measure accurately only the particular function investigated. Generally, malts of high saccharogenic activity have high dextrinogenic activity. However no prediction of relative liquefying power may be made from either of these activities.

Accurate estimation of the amounts of the individual alpha- and beta-amylases in malt is possible only by the use of methods designed specifically for the purpose. While saccharogenic values are largely dependent on *beta-amylase* and therefore give some idea as to relative content of this component, *alpha-amylase* may be estimated only by the use of methods in which varying content of beta-amylase does not influence the results. Present evidence indicates that either the starch liquefaction method of Józsa and Johnston (1935) or the modified Wohlgemuth technique of Sandstedt, Kneen, and Blish (1939) is specific for the alpha component. In view of the present uncertainty regarding the exact relationship between starch liquefaction and starch dextrinization it is suggested that the liquefying power of alpha-amylase be termed " alpha liquefying activity " and the dextrinizing power of this component termed " alpha dextrinogenic activity."

It appears that the two most significant of the amylolytic determinations commonly made on malt are the *malt saccharogenic activity* and the *alpha dextrinogenic activity*. From these two determinations the beta-amylase activity may be calculated. The summation of the alpha- and beta-amylase activities provides an approximation of the malt dextrinogenic activity. Alpha-amylase values alone accurately measure starch-liquefying power, provide an estimation of the ability of the malt extract to hydrolyze raw wheat starch, and permit conclusions as to the probable autolytic diastatic activity of the malt meal itself. It would seem then that any description of the amylolytic power of a malt must include at least values for these two activities: malt saccharogenic activity and alpha-amylase activity.

Two conversion factors were calculated and are tentatively proposed:

Malt saccharogenic units (Kneen and Sandstedt, 1941) $\times$ 7.5 = degrees Lintner.

Alpha dextrinogenic units (Sandstedt, Kneen, and Blish, 1939), $\times$ 19.0 = starch-liquefying activity in liquefons per gram (when the reaction temperature is 30°C for both).

Fairly good agreement was found between the amylolytic activities of the total extracts and the corresponding activities of the free extracts. In general then, malts high in " free " amylase are usually high in " total " amylase. However individual variation makes any attempt at accurate prediction unwarranted.

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PARALLELISM BETWEEN STARCH DEXTRINIZING .AND LIQUEFYING ACTIVITIES OF AMYLASES [1, 2]

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Three types of activities have been noted when boiled or gelatinized starch is treated with diastatically active enzymes such as are found in malt and other biologically active substances: (1) saccharification, characterized by a progressive development of reducing sugars, chiefly maltose and glucose, (2) dextrinization, characterized by the conversion of the starch to dextrins, which is denoted by a loss of the starch's characteristic property of turning blue when treated with iodine, and (3) liquefaction, indicated by a rapid decrease in the viscosity of the starch paste.

Although little is known of what takes place chemically when starch paste is liquefied by malt, it is not unreasonable to suppose that this liquefaction, like saccharification and dextrinization, is also a manifestation of the hydrolytic cleavage of the starch molecule. Thus, either the action of the dextrinizing component of malt amylase, *alpha-amylase,* or the saccharifying component, *beta-amylase,* or of both acting together, might reasonably be expected to contribute to the liquefying process Some investigators believe, however, that this liquefaction is independent of both dextrinization and saccharification and the possibility of an independent *liquefying component* has been pointed out. Chrzaszcz and Janicki (1932) present a summary of the work pertinent to this question up to the year 1932.

Later Waldschmidt-Leitz and Mayer (1935) reported the isolation of a distinct liquefying amylase from barley malt. These authors designated this component as an *amylophosphatase,* and attributed its liquefying activity to a disaggregation caused by the splitting off of esterified phosphorus from the starch aggregates. Friedmann (1939) disagreed with these authors and found that phosphorus was not liberated upon liquefaction. Mayer (1939) showed that starch which has been treated with a low concentration of iodine is resistant to saccharification but is still liquefied by an amylase preparation. Lüers and Rümmler (1935) claimed that the destruction of the saccharogenic amylase by heating an extract of malt to 70°C for 15 minutes had little effect on liquefying power, but decreased dextrinization markedly. Samec and Dolar (1939) showed that the initial change of liquefaction of a starch paste is independent of the change in reducing power or iodine color. These changes were thus supposedly related to different partial processes in the degradation of the starch molecule. On the other hand Józsa and Johnston (1935) and Blom, Bak, and Braae (1937) believe that liquefaction is chiefly attributable to the activity of alpha-amylase, and that this function can be measured quantitatively by viscometric procedure. Friedmann (1939) reported that *both* the dextrinizing and saccharifying amylases contribute to the liquefying process.

The various methods for determining the nature and degree of starch degradation by diastases have as their basis one or more of these three types of activities. Examples of the three different types of methods for following the amylolytic degradation of starch are: (1) the well known Lintner method and the Blish and Sandstedt (1933) method [4] for the saccharification of starch, (2) the Wohlgemuth (1908) and the Sandstedt, Kneen, and Blish (1939) methods for the dextrinization of starch, and (3) the Józsa and Gore (1930) and the Blom and Bak (1938) methods for the liquefaction of starch.

In the interests of improved technological control in the cereal industries, with special reference to baking, to malting, and to other industrial activities involving starch degradation, there is a recognized need for more certain knowledge as to the relative selectivities and values of the various types of methods available for measuring amylolytic processes and for interpreting their manifestations. Is it indeed true that the liquefaction and dextrinization of starch pastes are both *attributable* to the action of a single enzyme, *alpha-amylase?* If this is so, which of the two types of procedure suitable for the estimation of alpha-amylase activity, *i.e.,* the Wohlgemuth type of method or the viscometric method, is preferable and why?

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[4] Modified to measure maltose production using an amylolytic active extract and a starch paste substrate.

The present report deals with studies undertaken in an attempt to obtain further information about the foregoing and related matters. The principal method of approach in these studies has been to note the presence or absence of a *parallelism* between liquefying and dextrinizing properties, using amylase preparations from different sources. This approach, which is similar to that adopted by Blom, Bak, and Braae (1938), is based upon the supposition that if the two functions are affected to an equal degree by alterations in temperature, pH, salt additions, etc., it is highly probable that only one enzyme is the factor involved. Lack of such parallelism in these respects would presumably indicate that more than one biocatalyst enter into the process.

Amylases were selected from three widely different sources: malted wheat, *Aspergillus oryzae* (takadiastase, commercial powder, Parke Davis Co., Detroit), and a bacterial preparation (commercial powder, Wallerstein Co., New York).

Methods

Liquefying activity: The viscometric method as proposed by Józsa and Gore (1930) was used for the determination of the liquefying activities of the amylases. This method was selected mainly because of its simplicity and adequacy for the purpose of this investigation.

A 100-ml pipette was used as the viscometer. This pipette was surrounded by a water jacket through which water at 30°C was circulated. The delivery tube of the pipette was marked 6 cm below the bulb and the time of flow from mark to mark of an 87.5% glycerol-water solution (sp gr 1.2213 at 30°) was 161 to 162 seconds at 30°C. The outflow time for water at that temperature was 36 to 37 seconds. The pipette was attached to an aspirator in such a manner that it could be filled or emptied at will by turning a two-way stopcock in the line between the pipette and aspirator.

The method of preparation of the standard starch paste was essentially the same as that used by Józsa and Gore (1930). A 4.36% (moisture-free basis) potato-starch paste was used but a detailed description of its preparation is omitted here since the modifications from the method cited were merely adaptations of the type of pasting containers, the high-speed stirrer, and the pipette viscometer used. The method cited was also modified to the extent that all the reactions were run and viscosity measurements were made at 30°C instead of 21°C. The standard paste flowed from the pipette viscometer at this temperature in 165 seconds with a deviation of 5 seconds between different preparations.

Dextrinizing activity: The Wohlgemuth procedure was used for the determination of the dextrinizing activities. The dextrinizing activity of an amylase preparation such as malt, which contains both alpha- and

beta-amylase, is a combined function of both types of amylases. In the following report a distinction is made between dextrinization and *alpha dextrinization* to the extent that the latter term is applied to the dextrinization due solely to the alpha-amylase of malt or of any other amylase preparation in which both types of enzymes are present.

Alpha dextrinizing activity: The method of Sandstedt, Kneen, and Blish (1939) was used. In this method an excess of beta-amylase is added to eliminate the effect of this factor in the dextrinization process. With this excess of beta-amylase present, the rate of production of starch degradation products, which give the standard iodine coloration, is dependent only on the activity of the alpha-amylase present in the enzyme preparation added.

Experimental

Determination of a liquefying curve: A curve showing the effect of liquefaction upon the outflow time of the paste was determined. Several lots of starch paste were prepared and different amounts of unliquefied and fully liquefied paste were mixed and the outflow time recorded. The fully liquefied starch was obtained by adding 15 ml of a concentrated malt extract to 150 g of the paste, allowing it to act for 30 minutes, and then boiling the paste for 3 minutes to inactivate the enzyme.

The results are shown in Figure 1. Each point on the curve represents an average of several determinations.

Effect of malt concentration on liquefaction: A typical curve showing the effect of various amounts of an amylase preparation upon the amount of starch liquefied is shown in Figure 2. The extracts from the various amounts of malted wheat flour were added to 150 g of the standard starch paste and the viscosity measured at the end of one hour. The amount of starch liquefied was then determined by referring to the curve in Figure 1.

Determination of the effect of beta-amylase upon liquefaction: An effort was made to determine the extent to which beta-amylase affected the liquefying process observed with malt. The liquefying activity of a flour extract (ungerminated soft wheat), which supposedly contained little or no alpha-amylase, was determined. It was observed that compared to malt wheat flour, over 160 times by weight as much unmalted flour was necessary to give the same amount of liquefaction. The wheat-flour extract showed a very high beta-amylase activity (measured by the rate of conversion of a starch paste to maltose by the Blish and Sandstedt ferricyanide method). The alpha-amylase activity of this extract was found to be very slight since it failed to give a detectable change in blue iodine coloration between a 1-hour and a 24-hour period of action upon a starch paste.
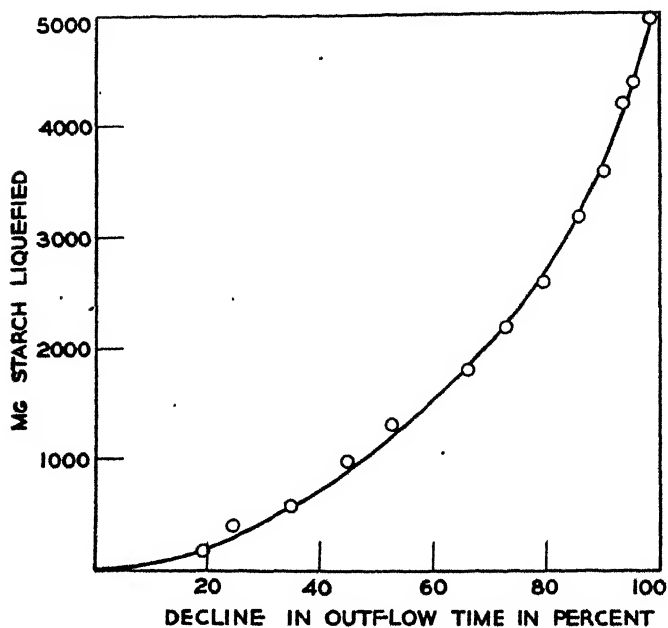
Fig. 1. Decline in outflow time with amount of starch liquefied.
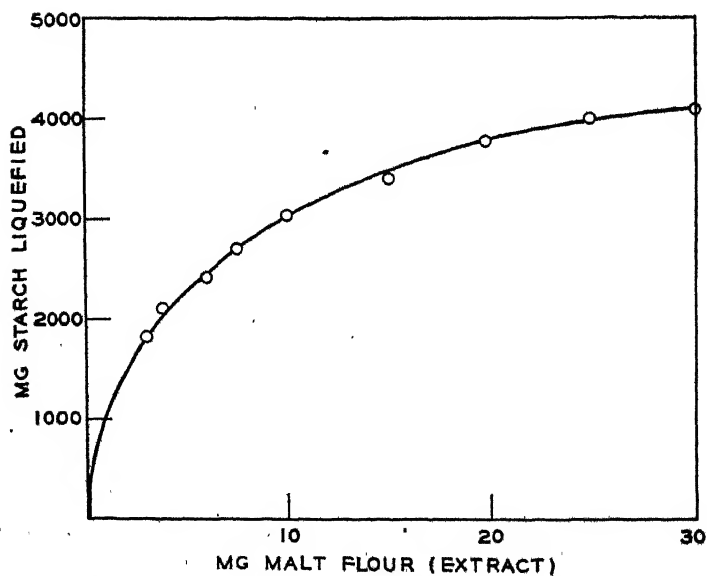
Fig. 2. Amount of starch liquefied with various amounts of malt flour.

It is shown by the data of Table I that the beta-amylase present in the small quantity of malt used for rapid liquefaction of the paste has a negligible effect in the liquefying activity of the malt. A malt extract was heated after the method of Ohlsson (1930) (70°C for 15 minutes) to inactivate the beta-amylase present. The percent of the original alpha-amylase which remained after this treatment was then determined. It was found that 84% of the original alpha-amylase activity remained. From this it was calculated that 1.2 ml of the heated preparation contained the same amount of alpha-amylase as 1.0 ml of the unheated, but the unheated contained a large amount of beta-amylase compared to the heated. The liquefying activities of these preparations were then compared in this ratio: 1 of the unheated to 1.2 of the heated. Closely corresponding outflow times would indicate that the beta-amylase present in the small amount of malt used did not affect the liquefaction.

TABLE I

COMPARISON OF THE LIQUEFYING POWERS OF HEATED AND UNHEATED MALT EXTRACTS, WHICH CONTAIN THE SAME AMOUNT OF ALPHA-AMYLASE

Malt extract concentration in 1,000 ml solution		Outflow time	
Unheated malt	Heated malt	Unheated malt	Heated malt
ml	*ml*	*sec*	*sec*
1.0	1.2	78.8	78.2
1.2	1.5	72.2	73.2
2.0	2.4	65.0	63.8
5.0	6.0	50.8	50.8
10.0	12.0	42.6	44.6

Determination of the extent of dextrinization occurring during liquefaction: An indication of the amount of dextrinization occurring during liquefaction can be obtained by comparing the time necessary for a liquefied or partially liquefied starch substrate to show the loss of blue iodine coloration, when acted upon by alpha-amylase, with the corresponding time for a nonliquefied portion.

Several 150-g samples of the standard starch paste were treated with 15 ml of a malt solution containing the extract of 40 mg of malt. At selected time intervals 10-ml portions of these samples were taken out and added to 10 ml of hot water and the mixture placed in a boiling water bath for 10 minutes. After cooling to 30°C each portion was treated with 10 ml of an alpha-amylase solution and the reaction time necessary to reach the standard iodine end-point was determined. The data given in Table II suggest that the amount of dextrinization occurring during the process of liquefaction is quite small.

Assuming 20 minutes to be necessary for the complete dextrinization

TABLE II

DETERMINATION OF THE EXTENT OF DEXTRINIZATION OCCURRING DURING VARIOUS STAGES OF THE LIQUEFACTION PROCESS

Time intervals	Time of outflow of paste	Decline in outflow time	Dextrinizing time	Approximate amount of dextrinization
min	sec	%	min	%
0	167.0	0.0	20.0	0.0
15	50.4	89.7	19.5	2.5
30	43.8	94.8	19.5	2.5
45	41.6	96.7	19.5	2.5
60	40.6	97.3	19.0	5.0
120	38.0	99.4	19.0	5.0

of the 10-ml aliquots, it was found that only about 5% or less of the dextrinization occurred during the liquefaction. This would indicate that the liquefaction is largely a preliminary step which occurs before very much of the starch is actually converted into dextrins. This conclusion agrees with that of Samec and Dolar (1939).

Comparison of the liquefying powers of different amylase preparations on the basis of equal dextrinizing activities: The concentrations of solutions of takadiastase, bacterial amylase, and malt alpha-amylase (heated malt extract) were adjusted until they showed equal dextrinizing activities as measured by the Wohlgemuth method. Using equivalent concentrations of these solutions, the liquefying powers were determined in order to note whether the adjustment to equal dextrinizing powers had also equalized the liquefying functions.

According to the preliminary determinations with the Wohlgemuth method it was found that the heated extract of 100 mg of the malt, 0.82 mg of the bacterial amylase preparation, and 3.0 mg of the takadiastase had equivalent dextrinizing activities. These amounts were dissolved in 100 ml of water and 15-ml portions of these solutions (150 g of starch paste) were used in the determination of the liquefying activity. The times of outflow of the starch paste at the end of 30 minutes were as follows: takadiastase 70.4 seconds, bacterial amylase 63.0 seconds, and alpha-amylase 64.0 seconds. If the dextrinizing and liquefying activities are functions of a single enzyme, the outflow times of the pastes would have been a constant value since the liquefying activities would have been equivalent.

The possibility arises that perhaps a small amount of a beta-amylase type component in the takadiastase accounted for the lack of parallelism between the two activities in the case of this preparation. If the takadiastase contained an amylase similar to beta-amylase along with its alpha-amylase type, this enzyme would probably increase the dextrinizing activity as measured by the Wohlgemuth method, and consequently the

liquefying value of the amount showing the same dextrinizing activity as the other two enzymes would be lower. It is noted that the viscosity of the paste liquefied by the takadiastase is higher (liquefying activity of the amylase lower) than in the cases of the other two enzymes. This fact was further substantiated by a comparison of the two types of activities as in the preceding experiment but unheated malt extract, known to contain active beta-amylase, was substituted for the heated preparation (alpha-amylase). The liquefying activity of the malt showed a decided deviation from that of the other two preparations, since the times of outflow of the starch were 85.0 seconds for the malt, 70.4 seconds for the takadiastase, and 63.0 seconds for the bacterial amylase.

Better correlation between the two types of activities was obtained by eliminating the beta-amylase factor in dextrinization by the use of alpha dextrinizing values in place of dextrinizing values. For example it was found that the outflow times of the starch liquefied by equivalent alpha dextrinizing concentrations were: takadiastase 48.2 seconds, malt 46.0 seconds, and bacterial amylase 44.8 seconds.

Allowing for all the errors encountered in the two methods, it can be concluded that for solutions showing equal alpha dextrinizing activities, a reasonably close agreement exists between their liquefying values.

Parallelism in the effects of temperature changes on liquefying and dextrinizing activities: As boiling temperature is approached, amylase activity is rapidly destroyed. If dextrinization and liquefaction are both functions of the same enzyme, the destructive effect of heat on one should parallel the effect upon the other. This type of parallelism was investigated for all three of the amylase preparations. These studies were made both with and without the presence of $CaCl_2$ and NaCl, in view of the fact that the calcium ion is known to greatly increase the resistance of amylases to heat inactivation (Wallerstein, 1908; Nakamura, 1931).

Bacterial Amylase

Solutions of the bacterial amylase were heated to different temperatures and the extent of inactivation measured by the viscometric and the Wohlgemuth-type methods. Each of three 50-mg samples of the bacterial amylase powder was dissolved in 200 ml of water, 200 ml of $0.05N$ NaCl solution, and 200 ml of $0.05N$ $CaCl_2$ solution, respectively. Three 50-ml samples of each of these solutions were heated at 50°, 60°, and 70°C, respectively, for 15 minutes.

The dextrinizing values were determined by using the equivalent of 2.5 mg of the amylase (heated and unheated) to 20 ml of 2.5% potato starch (wet basis). The percent of activity remaining after these treatments can be calculated from these dextrinization values. The liquefying values were determined by using the equivalent of 0.1875 mg of the

amylase to 150 g of the starch paste. The viscosities were measured at the end of 30 minutes. The percent of liquefying activity remaining after the heat treatment can be calculated by means of the curve in Figure 3. The results are shown in Table III.
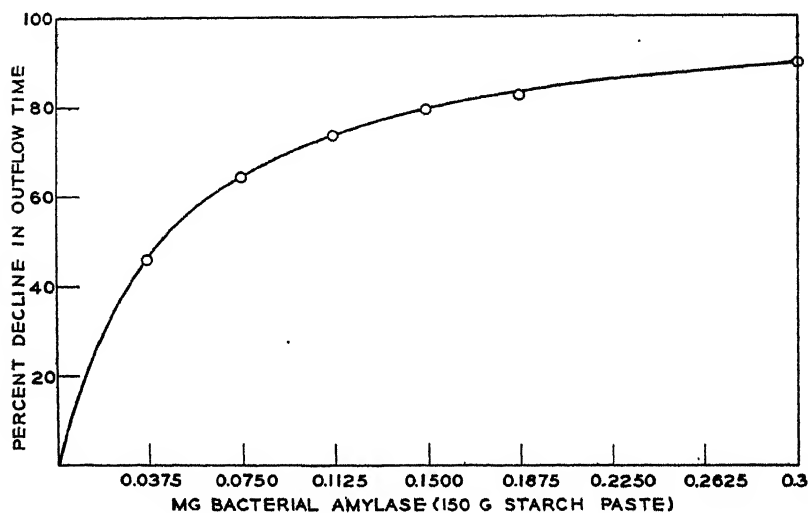
Fig. 3. Decline in outflow time of the starch paste when liquefied by various amounts of bacterial amylase.

TABLE III

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE BACTERIAL AMYLASE PREPARATION

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		min	sec	%	%
Unheated	H_2O	16.0	59.0	100.0	100.0
Unheated	NaCl	16.0	59.4	100.0	100.0
Unheated	$CaCl_2$	16.0	59.6	100.0	100.0
50	H_2O	18.5	61.0	86.5	90.0
50	NaCl	18.5	61.5	86.5	90.0
50	$CaCl_2$	18.5	60.8	86.5	90.0
60	H_2O	26.0	67.0	61.5	70.0
60	NaCl	29.0	71.0	55.0	60.0
60	$CaCl_2$	22.5	63.5	71.0	80.0
70	H_2O	120.0	114.4	13.3	15.0
70	NaCl	197.0	124.4	8.0	11.0
70	$CaCl_2$	40.5	78.0	39.5	47.0
75	$CaCl_2$	137.0	121.4	11.5	12.0

The correlation between the values in the last two columns in Table III would indicate that approximately the same degree of inactivation by heat is shown in the liquefying activity as in the dextrinizing activity. The calcium ion seems to serve as a protective agent to the same extent for both the liquefying and dextrinizing functions.

Takadiastase

The same procedure was followed with the takadiastase as with the bacterial amylase. Solutions containing 1 mg per milliliter of the powdered takadiastase were heated to the specified temperatures. The dextrinizing activity was determined by the use of 10 mg of the amylase preparations to 20 ml of the 2.5% starch. The liquefying activity was determined with 1.5 mg of the amylase to 150 g of the standard starch paste, and the reaction was allowed to run for 30 minutes. The data are given in Table IV. The percent of liquefying activity remaining after heating was calculated from the curve in Figure 4.

TABLE IV

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE TAKADIASTASE PREPARATION

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		*min*	*sec*	%	%
Unheated	H_2O	15.0	51.6	100.0	100.0
Unheated	NaCl	15.0	52.0	100.0	100.0
Unheated	$CaCl_2$	15.0	51.0	100.0	100.0
50	H_2O	19.0	56.2	79.0	68.0
50	NaCl	40.5	65.6	37.0	42.5
50	$CaCl_2$	15.0	51.4	100.0	100.0
60	H_2O	—	152.5	—	1.0
60	NaCl	—	158.4	—	1.0
60	$CaCl_2$	19.5	54.2	77.0	82.0
70	$CaCl_2$	—	148.6	—	1.0

The same tendency towards parallelism between the two activities was found for takadiastase as was demonstrated for bacterial amylase. The calcium ion again served as a protective factor against the heat inactivation to the same degree in both activities.

Malt Alpha-Amylase

Following the same procedure the two activities were compared by the use of malt alpha-amylase. Solutions containing alpha-amylase corre-
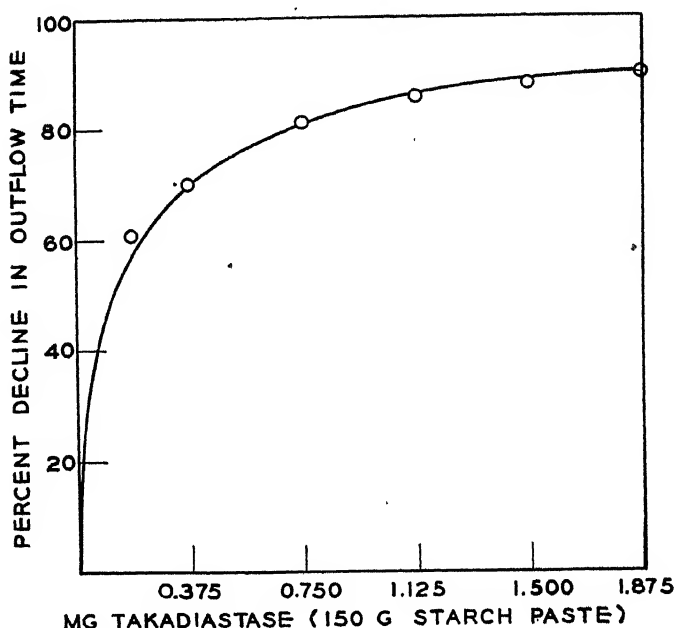
Fig. 4   Decline in outflow time of the starch paste when liquefied by various amounts of takadiastase

sponding to the extract of 40 mg of the original malted wheat flour per milliliter were heated to the specified temperatures. Of these solutions the amounts corresponding to 400 mg and 30 mg of malt were used for the determinations of the dextrinizing and liquefying activities, respectively. A continued close agreement existed between the two activities as evidenced by the data given in Table V. The percent of liquefying activity remaining in each preparation after the heat treatments was determined by the use of Figure 5.

Malt Extract

To recheck the belief that beta-amylase in malt affects the dextrinizing power but not the liquefying power of the malt extract in the concentrations used for each method, partial heat inactivation was carried out with and without the use of an excess amount of beta-amylase in the determination of the dextrinization values.

Concentrations of the malt extract equivalent to those used for the malt alpha-amylase in the preceding determination were used here. More dilute solutions of the malt extract were heated and used for the determinations of the alpha dextrinizing activity, since presence of the

excess of beta-amylase decreased the Wohlgemuth times about 80% for the same amount of malt.

TABLE V

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING
AFTER PARTIAL HEAT-INACTIVATION OF THE MALT
ALPHA-AMYLASE PREPARATION

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		min	sec	%	%
Unheated	H₂O	11.5	53.0	100.0	100.0
Unheated	NaCl	11.5	53.2	100.0	100.0
Unheated	CaCl₂	11.5	53.0	100.0	100.0
50	H₂O	13.0	54.0	88.5	90.0
50	NaCl	13.5	55.2	85.0	80.0
50	CaCl₂	12.5	53.8	92.0	91.0
60	H₂O	16.0	57.2	72.0	66.0
60	NaCl	18.0	60.2	64.0	56.0
60	CaCl₂	13.5	55.0	85.0	82.0
70	H₂O	—	120.5	—	5.0
70	NaCl	—	129.2	—	4.0
70	CaCl₂	23.5	63.0	49.0	50.0
75	CaCl₂	—	150.8	—	—
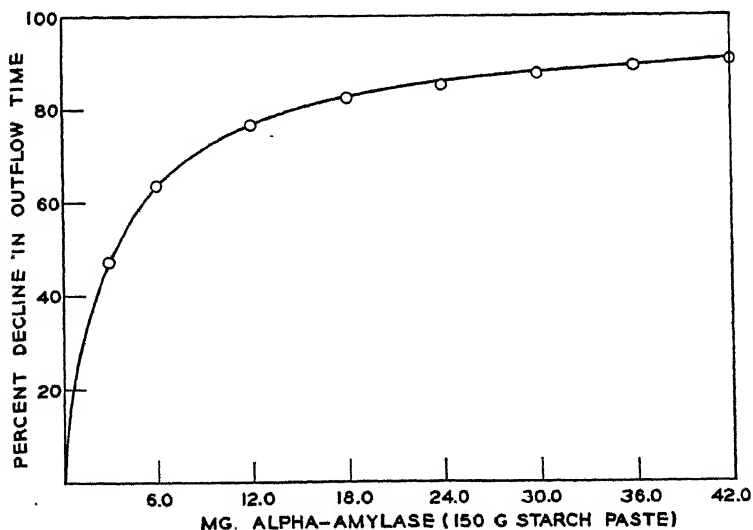
Fig. 5. Decline in outflow time of the starch paste when liquefied by various amounts of malt alpha-amylase.

The data obtained in the two instances are given in Tables VI and VII. The percent of liquefaction in each case is calculated by means of the curve in Figure 6.

The values for percent of activity as measured by the two methods show a much higher correlation in Table VII than in Table VI. Heating affected the amount of active beta-amylase present as well as the amount of alpha-amylase that remained active. With the low enzyme concentra-

TABLE VI

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE MALT EXTRACT

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		min	sec	%	%
Unheated	H_2O	9.5	50.2	100.0	100.0
Unheated	NaCl	9.5	50.6	100.0	100.0
Unheated	$CaCl_2$	9.5	50.2	100.0	100.0
50	H_2O	10.0	50.6	95.0	100.0
50	NaCl	11.0	51.8	86.0	90.0
50	$CaCl_2$	10.0	50.6	95.0	100.0
60	H_2O	16.5	53.4	57.5	80.0
60	NaCl	34.0	58.0	28.0	60.0
60	$CaCl_2$	20.5	50.8	46.0	100.0
70	H_2O	197.0	91.0	4.8	2.0
70	NaCl	—	150.4	—	—
70	$CaCl_2$	27.5	53.8	34.5	78.0

TABLE VII

DETERMINATION OF THE ALPHA-DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE MALT EXTRACT

Heat treatment (temperature)	Solution	Alpha dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		min	sec	%	%
Unheated	H_2O	18.5	50.2	100.0	100.0
Unheated	NaCl	18.5	50.2	100.0	100.0
Unheated	$CaCl_2$	18.5	50.4	100.0	100.0
50	H_2O	21.0	50.6	88.0	97.0
50	NaCl	28.5	54.6	65.0	68.0
50	$CaCl_2$	21.0	51.0	88.0	94.0
60	H_2O	31.0	57.6	60.0	61.0
60	NaCl	71.5	73.6	26.0	36.0
60	$CaCl_2$	22.0	51.4	84.0	91.0
70	H_2O	—	123.0	—	4.0
70	NaCl	—	·161.4	—	—
70	$CaCl_2$	42.0	61.4	44.0	52.0
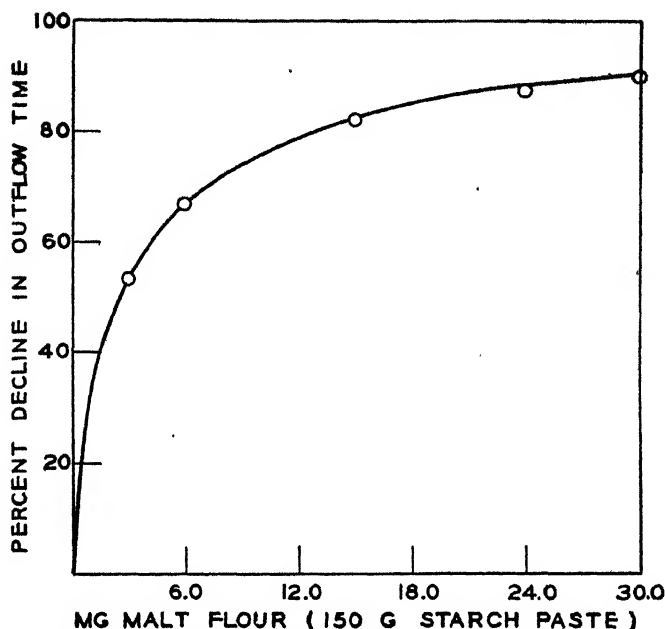
Fig. 6. Decline in outflow time of the starch paste when liquefied by unheated malt extract.

tion used for liquefaction only alpha-amylase was effective, whereas *both* alpha- and beta-amylase are effective in dextrinization. This would explain the results obtained by Lüers and Rümmler (1935) in which they showed that heating affected dextrinizing activity but had little effect upon liquefying activity. This would most likely explain also why these authors were unable to obtain correlation between these two activities in their partial inactivation of barley malt with acid and ultraviolet light. Very little correlation would be expected between the two activities as long as the partial heat-inactivation affected the beta-amylase and this amylase affected the iodine values but not the liquefying values. The elimination of this factor by the use of alpha dextrinizing values of the malt brought about better correlation, as is shown in Table VII.

It is noted that the amount of inactivation upon heating, as measured by the liquefying activity shown in Table VII, is greater than that shown in Table VI for the same conditions. This is no doubt due to the fact that more dilute solutions of the malt were heated in the cases using excess beta-amylase, and the amount of inactivation of the alpha-amylase of a malt solution upon heating is dependent upon the concentration of the malt extract heated.[5]

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[5] The effect of concentration of protein and the presence of various ions upon the heat inactivation of malt is being investigated in this laboratory.

Since the correlation between the dextrinizing and the liquefying activities after partial thermal inactivation is high for all three enzymes, the conclusion may be drawn that the two activities are functions of the same enzyme. The calcium ion is shown to be a protective factor against heat inactivation in all three cases and it shows the same amount of protective action towards both activities. This is further evidence that liquefaction and dextrinization are affected by the same amylase. The amylases heated in the presence of the sodium ion seem to be inactivated more than when heated in water alone.

Parallelism in the effects of changes in acidity on liquefaction and dextrinization: It has long been known that amylase activity is profoundly influenced by degree of acidity. Any variation from a certain optimum (dependent upon the amylase) produces a decided decrease in enzyme activity. All of the previous determinations were made at a pH of 4.7, which was chosen as an arbitrary and convenient working acidity. In this particular phase of the work the pH was varied and the effects of this variation on the liquefying and dextrinizing activities were determined. A tendency towards parallelism would be expected in agreement with the previous observations.

The pH was varied by means of buffer solutions with different ratios of acetic acid to sodium acetate. The total concentration of the acetate ion was kept constant. The same ratios of bacterial amylase, takadiastase, and malt alpha-amylase to starch were used as in the preceding studies of dextrinizing and liquefying activity.

The pH of each starch sample was determined before and after the enzyme treatment by use of the Coleman pH electrometer. It was found that the values sometimes varied slightly between the two determinations. This is most likely due to the difference between the buffering action of the starch and that of its degradation products. However, in the data found in Table VIII only the pH values before the enzyme action are given.

The results found for the effects of the change in the acidity upon the liquefying and dextrinizing activities of the three amylases are tabulated in Table VIII.

In general there is a strong tendency towards parallelism with this type of comparison. The correlation is much higher between the two activities in instances where the pH was not greatly different from the optimum.

The bacterial amylase showed a progressive decrease in activity as the hydrogen-ion concentration of the substrate was increased. Takadiastase and malt alpha-amylase showed comparatively less variation in activity in the pH range selected, since the optimum in both cases fell towards the intermediate pH values. Since the decrease in activity with

TABLE VIII

DETERMINATION OF THE RELATIVE LIQUEFYING AND DEXTRINIZING ACTIVITIES OF
THE THREE AMYLASE PREPARATIONS AT VARIOUS pHs

pH	Bacterial amylase		Takadiastase		Malt alpha-amylase	
	Dextrinizing activity	Liquefying activity	Dextrinizing activity	Liquefying activity	Dextrinizing activity	Liquefying activity
	%	%	%	%	%	%
6.1–6.2	100	100	81	75	75	81
5.2–5.3	95	95	100	100	100	100
4.9–5.0	88	94	100	100	100	100
4.4–4.5	70	84	96	92	95	97
4.2–4.3	50	70	93	89	93	90
3.4–3.5	—	10	72	70	20	28

variation of pH was not as great in the latter two instances, it is to be expected that the inactivation of the enzymes would not be as much of a factor, and higher correlation between the activities would be obtained.

Summary and Conclusions

The dextrinizing and liquefying functions of amylases from three different sources (malted wheat, *Aspergillus oryzae,* and a bacterial preparation) were studied. Malt dextrinizing power was measured by a Wohlgemuth iodine method, alpha dextrinizing activity by the method of Sandstedt, Kneen, and Blish (1939) and liquefying power by the viscometric procedure of Józsa and Gore (1930).

When extracts of the three enzyme preparations were adjusted to an equal basis in terms of alpha dextrinizing power their liquefying activities also were found to be substantially the same. The two types of activity appear to be equally affected by heat and by changes in pH. Calcium ions seem to protect both functions equally against heat inactivation. This consistent parallelism of behavior justifies the conclusion that both the liquefaction and the alpha dextrinization of starch paste by amylases are attributable to the action of *one enzyme,* alpha-amylase.

Since it appears that either the Sandstedt, Kneen, and Blish or the Józsa and Gore viscometric method may reliably be used for the estimation of alpha-amylase activity, it is appropriate to consider the distinctive features of each method, in the interests of establishing a preference for one or the other. A critical and somewhat undesirable feature of the viscometric method is the preparation of starch pastes having standard and constant properties. The mechanical treatment in the preparation of these starch pastes is a factor of vital importance, requiring considerable time and careful manipulation. For a specified duration of time the degree of liquefaction is not a linear function of the quantity of amylase present; hence a reference curve must be established and used for calculating results.

The modified Wohlgemuth iodine method is perhaps more convenient than the other one and more readily adaptable to general standardization. It is primarily a chemical, rather than a physical, method. It requires less equipment and is less time-consuming than the viscometric method. The time required to reach the iodine end-point is a linear function of the amount of amylase present. The Wohlgemuth end-point represents a far more advanced stage of starch degradation than is true of the values obtained in the viscometric procedure.

A notable difference between the methods arises from the fact that beta-amylase is an influencing factor in the Wohlgemuth method unless an excess is added as in the Sandstedt, Kneen, and Blish procedure. However, under the conditions and specifications found most convenient for the operation of the starch-liquefaction method, beta-amylase was not a factor of significance. This is due to the relatively far greater enzyme *dilution* that must necessarily be used in this method. If the concentrations used were equal to those specified for the Wohlgemuth iodine method it is quite probable that beta-amylase would be a factor influencing the rate of liquefaction, as was indicated by trials in which large amounts of beta-amylase were used.

Amylases from different sources may show certain differences in properties. Thus the amylase of bacterial origin shows a higher optimum pH range and a greater resistance to heat than do the amylases of malted wheat and of *Aspergillus oryzae*.

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FURTHER STUDIES ON THE RETARDATION OF THE STALING OF BREAD BY FREEZING

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Recent experiments on the freezing of bread by Cathcart and Luber (1939) showed that the development of an " off " aroma was the principal factor limiting the time that bread could be kept salable by freezing. Cathcart and Luber's work showed that bread in sharp freezers at — 22°C remains salable for about 40 days. Bread stored at — 22°C for longer periods was considered unsalable because of the development of this " off " aroma. Bread stored in freezers at temperatures higher than — 22°C developed this aroma sooner and that stored at lower temperatures developed it more slowly.

Cathcart and Luber pointed out two reasons which might account for the development of this aroma: activity of enzymes of microorganisms at low temperatures and oxidation of proteins and fats. The first cause is probably of little significance because of the high baking temperatures employed. The temperatures are such that they would inactivate the enzymes and destroy most microorganisms in the loaf, only a few spore formers being able to survive. Cathcart and Luber were also able to rule out any effect of absorbed odors from the papers used for wrapping, since the off aroma developed in unwrapped bread.

Also, the intensity of the aroma did not seem to be greater in the unwrapped samples, thus more or less eliminating the possibility of its being absorbed from the commercial freezer.

The following experiments were conducted in an attempt to determine the cause of the development of the off aroma, as well as to seek ways and means of eliminating it.

Quicker Freezing

In order to find out if quicker freezing methods would delay the development of this off aroma, one such method was tried. Commercial bread was frozen in a freezing tunnel at — 22°C and at — 35°C. In both cases wrapped and unwrapped breads were frozen. Moisture-proof waxed paper wrappings were used for wrapping all frozen bread. The freezing curves obtained by inserting thermometers into the center of the loaves, and reading them at intervals, are given in Figure 1. The
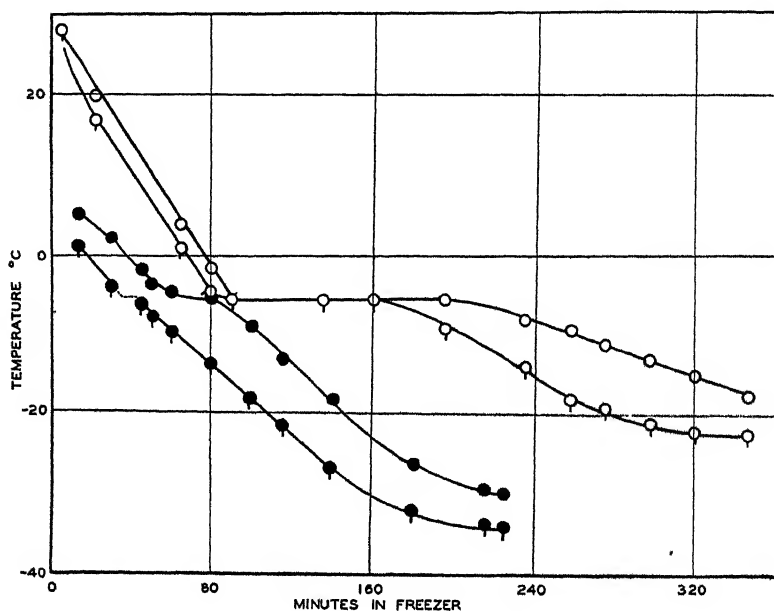
Fig. 1. Rate of cooling of bread in forced-air tunnel. Open circles represent wax wrapped, — 22°C; open circles with suspended bar, unwrapped, — 22°C. Closed circles represent wax wrapped, — 35°C; closed circles with suspended bar, unwrapped, — 35°C.

flat portion of the curve at about — 5°C represents the latent heat of freezing of the bread. The comparison of these curves with those reported by Cathcart and Luber (1939) shows that the wrapped bread in the tunnel froze faster than that in still air at the same temperature.

However, the unwrapped bread froze at still a faster rate, especially at a temperature of — 35°C.

The bread frozen at — 22°C was stored at — 22°C, while the bread frozen at — 35°C was divided into two portions, half of it being stored at — 35°C and half stored at — 22°C. Samples of the same bread were put into individual freezers (— 22°C and — 35°C) and frozen in still air. As soon as the unwrapped loaves were removed from the freezing tunnel they were wrapped (in the freezer) and the wrappers sealed. All the samples of bread were tested at intervals by the " swelling power " test and by two persons who were experienced in judging bread.

After 30 days in the freezer there was no significant difference either by the " swelling power " test or by organoleptic tests between the bread frozen in still air at — 22°C and stored at — 22°C, the bread frozen in the freezing tunnel at — 22°C and stored at — 22°C, and the bread frozen in the freezing tunnel at — 35°C and stored at — 22°C. Whether the loaves were frozen in a wrapped or an unwrapped condition made little difference. Tests at longer periods of time indicated that the off aroma developed at equal rates in all of these samples.

The samples frozen in the freezing tunnel at — 35°C and stored at — 35°C, whether frozen in a wrapped or an unwrapped condition, showed no advantage over wrapped bread frozen and stored in still air at — 35°C. As was shown by Cathcart and Luber, however, all samples stored at — 35°C kept longer than those at — 22°C.

The unwrapped samples frozen in the freezing tunnel showed some drying out. This, of course, is to be expected as a result not only of the unwrapped condition but of the forced draft in the freezing tunnel. All of the " swelling power " tests showed a decrease to a value characteristic of stale bread; then an increase, after 15 or 20 days, to a value of 38 to 40 ml in sediment; and then a decrease again to a value that would be expected for stale bread. This is mentioned since it confirms an observation reported by Cathcart and Luber (1939).

All of the tests and details are not presented since the quicker freezing did not affect the results significantly.

Effects of Ingredients and Antioxidants

In order to test the effect of ingredients, bread was made in accordance with commercial practice, and all ingredients were kept constant except the one to be tested. One set of control loaves was made in the normal manner with all the common enriching ingredients. Another set contained only flour, salt, yeast, and water. Another contained all the ingredients except dry skim milk, another all the ingredients except

shortening, and another all ingredients except malt extract. Other loaves were made with 1%, 2½%, and 5% of Avenex No. 7 (specially milled oat flour). Some of the control loaves were wrapped with a special glassine avenized (oat-flour-treated) paper before the moisture-proof wax was applied. All of the loaves were wrapped in wax paper in the commercial manner and put in a sharp freezer at — 22°C.

Samples of the various breads were taken from the freezer at intervals, peroxide values determined, and organoleptic tests made. The peroxide values were determined on residues obtained by extracting a ground sample of the bread with ether and evaporating off the ether on a water bath. The technique used was essentially that of Wheeler (1932). Every effort was made to handle the peroxide procedure in exactly the same way at all times so that the results would be strictly comparative. The organoleptic tests were again made by two persons experienced in judging bread. The loaves were tested at intervals for a period of three months after they had been placed in the freezer.

There was no significant difference, either in peroxide values or organoleptic flavor tests, between the loaves made with the normal formula (containing enriching ingredients), the lean formula, the one without milk, the one without shortening, and the one without malt. According to organoleptic tests the off aroma developed at about the same time in all of them.

Although the peroxide values on the loaves containing oat flour and those wrapped in the oat-flour-treated paper were slightly lower in general (with considerable variations) than the values on the normal loaves, the organoleptic tests did not show significant differences. According to the scorers, the development of the off aroma came at about the same time, whether the loaves contained oat flour, were wrapped in oat-flour-treated paper, or were simply standard loaves wrapped in moisture-proof wax wrappers. Thus, since neither variations in formula nor the use of oat flour as an antioxidant made any significant difference in the results, the detailed data are not presented.

Canning of Bread

Since the above results gave some indication that oxidation might at least be one factor in the development of the "off" aroma, it was decided to freeze and store some bread that had been sealed hermetically. Three samples of commercial bread were used: one sample was sealed in wax wrappers, one was sealed air-tight in a standard commercial tinned can, and the third was vacuum packed in a tinned can. The bread was packaged three hours after being baked. It was then immediately placed in a sharp freezer at — 22°C and stored. Samples were

tested at various periods of time by the " swelling power " test and by organoleptic flavor tests.

These tests are summarized in Table I. Since the results on the control loaves wrapped in wax paper were the same as those reported by Cathcart and Luber (1939), the tests on these loaves are not included in the table. It will be noted from the table that both samples remained in good condition and were salable up to the 345-day test. At 565 days both samples showed a decrease in quality, the crumb color becoming darker and more damp to the touch. However, even at 565

TABLE I

Tests on White Bread Canned and Frozen at − 22° C
for Various Periods of Time

(Bread was three hours out of oven when canned and placed in freezer.
Four hours allowed for thawing before opening and testing.)

Series No.	Time in freezer	Canning process	Swelling power	Score		Remarks
				Aroma 15	Taste 20	
1	*days* 21	Hermetic Vacuum	34 34	14.0 14.0	19.0 19.0	All characteristics same as fresh bread.
2	60	Hermetic Vacuum	32 32	13.5 14.0	19.0 19.0	Crust character and aroma slightly better on vacuum sample. Vacuum sample same as fresh bread.
3	160	Hermetic Vacuum	39 35	13.5 14.0	19.0 19.0	Crust character and aroma slightly better on vacuum sample. Vacuum sample same as fresh bread.
4	345	Hermetic Vacuum	34 36	13.5 14.0	19.0 19.0	Vacuum sample slightly better. Aroma slightly stronger on both samples.
5	565	Hermetic Vacuum	34 34	12.5 13.0	18.0 18.5	Both samples showed a decrease in quality. The crumb color of both was dull.

days there was little evidence in either sample of the off aroma that had developed in the uncanned bread. The comments and remarks of the judges show that the sample packed in vacuum was slightly preferable to that simply sealed in a can at normal atmospheric pressure. The " swelling power " values showed little variation throughout the test; however, there is an indication of an increase in the values at the 160-day test.

These experiments on the canning of bread indicate that the development of the off aroma is at least to some extent due to oxidation, and

that by preventing this oxidation (in this instance by canning), the salability of bread frozen and held at — 22°C can be increased from about 40 days to at least 345 days. The process of vacuum canning the bread before freezing not only increases the length of time that the bread remains salable but keeps the bread in excellent condition for a period of about one year.

Since quick-frozen foods in cans are showing possibilities of success, it may be that the canning and freezing of bread for storage over long periods of time and for special purposes will prove practical in the future. In commercial practice the canning of bread is not new. There have been references to canned bread in the bakery trade journals for the past fifteen years. The most recent and comprehensive report on the canning of bread was given by Pfannenstiel and Salomon (1939). They did not stress the keeping qualities of the bread but noted that when the bread was baked in air-tight cans it was crustless, and the crumb had the same properties, including moisture content, as ordinary bread. They pointed out that the acidity was higher, the vitamin content higher, that complete sterilization was attained, and that consumer tests gave highly satisfactory results.

Published reports generally are concerned with the baking of bread in cans and preservation at room temperatures after baking. Most workers claim a long keeping time for bread baked by this process. Experiments conducted here at the Institute indicate that such bread would have a long keeping time as far as its moisture content is concerned, but staling, due to starch conversion, goes on very rapidly. Thus the bread loses its freshness as judged organoleptically and becomes more solid, just as it would if it were in a wax wrapper (this was verified by experiments). However, if bread were baked in the can and then frozen, it would undoubtedly keep in excellent condition for at least a period of one year, and perhaps longer, on the basis of results reported above. Of course the advantage of the procedure as carried out in this work is that it produces bread that is identical with commercial bread as offered to the trade.

Summary

The following methods have been used in attempting to increase the length of time that bread can be kept fresh and salable by freezing at — 22°C or — 35°C by sharp freezing methods as employed by Cathcart and Luber (1939): (1) quicker freezing by employing a freezing tunnel, (2) variations in formula and use of oat flour as an antioxidant, (3) hermetically sealing in the normal atmosphere and in vacuum, employing modern canning technique, before freezing.

The quickest method of freezing employed reduced the bread to the solid state (internal temperature — 10°C) in 60 minutes. This represented a reduction in freezing time of at least 60 minutes over the quickest method employed by Cathcart and Luber in their sharp freezing experiments. However, the quicker freezing method did not show a significantly longer keeping time for the bread than sharp freezing at the same temperature.

Variations in the formula or the use of oat flour as an antioxidant (directly in the product or on the wrapping paper) did not lengthen the keeping time significantly according to organoleptic tests, although oat flour showed a slight lengthening of the keeping time according to peroxide values.

The baking and subsequent canning (hermetically sealed in normal atmosphere and in vacuum) gave the bread a greatly increased keeping time. The bread sealed in the normal atmosphere remained salable for a period of approximately one year, while that in the vacuum not only remained salable but remained quite similar to fresh bread for this same period.

From the practical standpoint of commercial bread production in the United States at the present time, this lengthening of the keeping time of frozen bread may not be of great importance. In fact, it is felt that the keeping time reported by Cathcart and Luber for frozen bread in moisture-proof wrappers is sufficient. Actually, however, it throws light on the cause of the off aroma that develops in frozen bread and should help to clarify the cause of so-called " stale " aroma that develops in time in ordinary bread that is not frozen. The canning and freezing of bread may also prove of value in cases of emergency.

Acknowledgment

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A COMPARISON OF HARD RED SPRING AND HARD RED WINTER WHEATS [1]

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(Read ·at the Annual Meeting, May 1940)

The relative baking qualities of hard red winter and hard red spring wheats has long been a subject of much debate, and little agreement. It has been rather generally accepted abroad that the hard red spring wheats are superior to the hard red winter wheats and the persistent spread in prices of the two classes of wheat and of flours milled from them has tended to establish this belief. In a study of published data relevant to this subject, Larmour (1940) concluded that the data presented did not warrant this conclusion. There was abundant evidence that the two classes differ in certain characteristics, but in regard to intrinsic baking quality there was no consistent support of the idea that one class is superior to the other.

It is possible, and indeed likely, that the experimental methods applied in studies of these two classes of wheats were inadequate to evaluate certain factors that a commercial baker might be able to recognize but which he could not describe quantitatively. Fisher and Jones (1937) in discussing the characteristics of the wheats of commerce, speak a good deal of the " tenderness " of doughs, a quality which is recognizable by the practical baker, but one which has thus far defied all attempts at accurate measurement. In describing flours milled from American hard winter wheats, they state that " such flour has a high water absorption but not so extensive a fermentation tolerance as that from Marquis, and though giving an excellent loaf when baked alone, has less carrying power for weak flours." They regard the hard winter wheat, as received in Great Britain, as being in the same category as the Plate wheats from Argentina; that is, they neither add to nor detract from the quality of a blend, and are therefore to be regarded as " filler " wheats.

A great many people in the United States, especially those in the southwest who have the opportunity to get the wheats and flours at first hand from the areas of production, do not agree that this is a correct and general description of American hard winter wheats. They think that the hard winter wheats procured from areas that are suited climatically to produce high-protein wheat make flour equal in baking qual-

ity to the spring-wheat flours. Indeed there are some who would go as far as to maintain that the hard winter wheat flours are actually superior to all others for commercial bakeshop practice. Larmour, Working, and Ofelt (1939) reported the results of baking tests made with the principal varieties grown at that time in the southwest. While no direct comparison with spring-wheat samples of comparable protein content was made, it was clearly evident from their data that the relation between loaf volume and protein was linear, and that the volumes were of the same order as would be expected from hard red spring wheat flours of comparable protein. It was thought at the time that this might have been attributable to the baking formula used, a rich formula with shortening and milk, which might have exerted a bolstering effect on the flours. It was decided to investigate this point at the earliest opportunity with both hard winter and hard spring flours. In the fall of 1939 some hard winter samples were procured from Kansas through the courtesy of Dr. J. H. Parker, and these were compared with a number of typical hard spring varieties grown in Saskatchewan, and selected on the basis of previous baking results. Both lots were of the 1939 crop.

Experimental

All the samples were milled to a straight-grade flour of approximately 70% extraction, on a three-stand Allis-Chalmers experimental mill. After aging for five weeks, the flours were baked by six baking formulas. Formula I was the " malt-phosphate-bromate " formula, consisting of the A.A.C.C. basic formula plus malt extract 0.3%, ammonium dihydrogen phosphate, 0.1%, and potassium bromate 0.001%, with standard fermentation, proof, and pan times, and temperatures. Formula II was a rich formula with yeast 3%, sugar 5%, salt 1.75%, shortening 4%, dry milk solids 4%, and malt, phosphate and bromate as in Formula I. The fermentation time was the same as above, but the pan proofing was done at 35°C for 55 minutes. Baking times and temperatures were the same for both formulas and were those set forth in the standard method. Four modifications of Formula II were used: no bromate, 2 mg, 3 mg, and 4 mg.

All doughs were mixed in a Hobart mixer equipped with two hooks; each 100-g batch was mixed separately. They were mixed at slightly variable times, until a smooth dough was obtained. All punching was done by means of National " pup " sheeting rolls. In panning, the doughs were first passed through the sheeting rolls twice and then rolled up by hand. Low-form tins were used for baking.

The baking results obtained by the malt-phosphate-bromate formula are given in Table I. The relation of loaf volume to protein content of the flour is shown graphically in Figure 1.

TABLE I

BAKING DATA FOR HARD RED SPRING AND HARD RED WINTER WHEAT VARIETIES
1939—MALT-BROMATE-PHOSPHATE FORMULA

Variety	Flour protein	Absorp- tion	Loaf volume	Tex- ture	Crumb color	Baker's comments
	%	%	ml	score	score	
1 Thatcher	16.7	66	1210	7 open	8.5	
2 Thatcher	14.9	67	1050	6 open	8.5	
3 Thatcher	13.6	66	880	8	8.0	
4 Thatcher	11.8	66	750	8	8.0	
5 Thatcher	10.8	66	695	8	8.0	
6 Renown	15.8	66	985	8.5	7.5	Renown samples all quite open.
7 Renown	14.4	64	915	7.5	7.5	
8 Renown	13.2	64	815	6	7.5	
9 Turkey	12.7	62	750	7	5.0	
10 Kanred	15.2	63	850	7.5	6.0	
11 Tenmarq	15.0	65	1010	7	7.5	Strong flour.
12 Blackhull	13.4	62	820	7.5	7.5	
13 Superhard Blackhull	15.1	65	740	7.5	7.5	
14 Chiefkan	11.3	63	580	4	6.0	Runny.
15 Cheyenne	14.5	64	900	9	9.5	Strong flour.
16 Nebred	15.4	64	1165	7	8.5	Strong flour.
17 Kawvale	9.6	60	570	5	6.0	
18 Clarkan	11.3	56	635	4	7.5	Short, dead.

It should not be regarded as surprising that the hard red spring wheat flours exhibit such linearity of loaf volume in relation to protein content, because these samples were selected from a large number previously baked, and they were chosen from the regression line of loaf volume on protein. They may be regarded as being very typical of Saskatchewan samples of the crop of 1939. The hard red winter samples, on the other hand, were single samples chosen virtually at random, and therefore would not be expected to show as direct a relationship between loaf volume and protein as the others even if they were all of one variety. Actually there were eight different varieties of hard red winter wheats and two soft red winter wheats in this series.

The two varieties of spring wheat were chosen because these two varieties, Thatcher and Renown, represent roughly the range of differentiation allowable for varieties in western Canada. It is well recognized that Thatcher is a very high quality variety, stronger than Marquis on the whole, but in view of the fact that it does not represent more than 50% of the total hard spring wheat production in Saskatchewan, it is scarcely to be considered fully representative of the spring wheat of western Canada at present.

Figure 1 shows that four of the ten winter-wheat varieties were distinctly inferior to the hard red spring wheat flours in loaf volume by the malt-phosphate-bromate formula. These four included Kanred, Super-

hard Blackhull, Chiefkan, and the soft wheat Clarkan. The others, Turkey, Tenmarq, Cheyenne, Blackhull, Nebred, and Kawvale, appeared to fall into places that would be expected from consideration of the hard
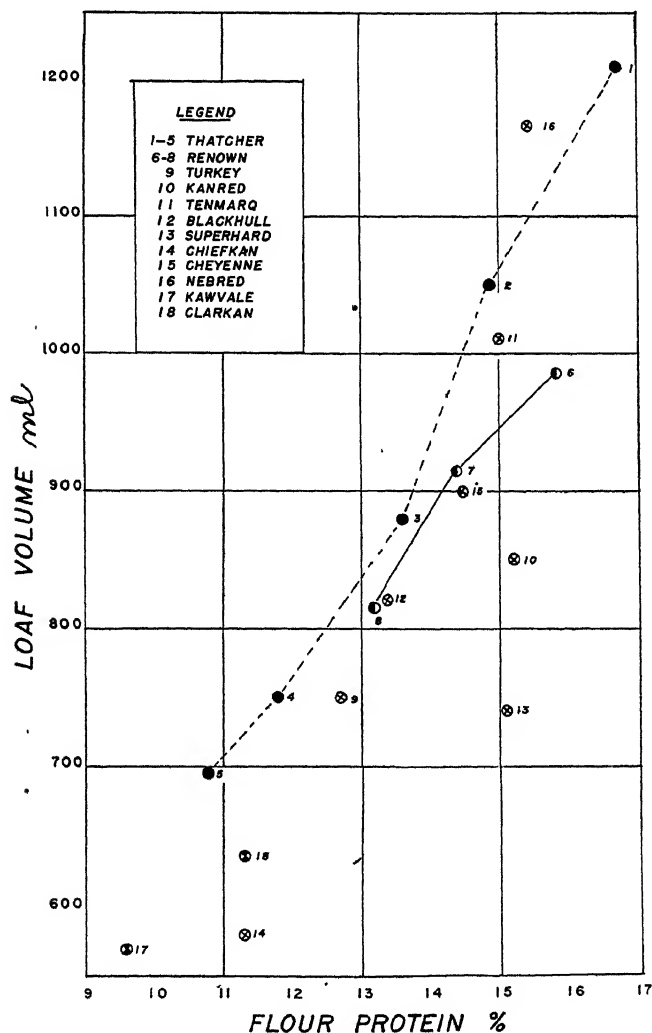
Fig. 1. Relation of loaf volume to flour protein, Formula I.

spring wheat samples. This was rather surprising because Formula I was developed particularly to show strength in the spring wheats, and from *a priori* consideration would hardly be expected to be well adapted

to evaluating another class of wheat which differed essentially in characteristics. However, it may be recalled that Bayfield used Formula I with considerable satisfaction in the evaluation of the soft wheats of Ohio.

With regard to other characteristics, it may be mentioned that the baker commented that the doughs from Tenmarq, Cheyenne, and Nebred felt and behaved during fermentation like " strong " flour doughs, by which is meant that they handled like the spring wheat flour doughs to which he had been accustomed. Careful examination of the crumb texture and grain failed to reveal any outstanding or describable differences between the two classes of wheat represented, except in the cases of the two soft wheats and Chiefkan, all of which were of coarser and harsher texture than the others.

It has to be concluded from this baking test that five of the eight hard winter wheat varieties were equal in intrinsic baking quality to hard red spring wheats of corresponding protein content, and that three were conspicuously inferior.

These flours were next baked by the rich Formula II, with various amounts of bromate. Ofelt and Larmour (1940) showed that the hard winter wheat flours naturally require more bromate than the hard spring flours, and it is well recognized that they need stronger dosages of Agene in commercial practice. It was thought desirable therefore to use a range of bromate dosages in order to get the optimum conditions for each flour in the series. The baking data for these bakings are given in Table II. The loaf volumes obtained at optimum bromate dosage are plotted against the protein content of the flour in Figure 2.

It can be seen in Table II that the optimum bromate dosage for Thatcher was 1 mg, for Renown and for most of the hard winter wheats it was 2 mg, although on the whole the differences between loaf volumes obtained with 1 and 2 mg of bromate were small and probably were not very significant.

In Figure 2 it is shown that the positions of Chiefkan and Superhard Blackhull were about as they were with Formula I; in respect to the rest of the samples they are both far below the others. The Kanred showed to better advantage, but the Cheyenne appeared poorer with Formula II than with Formula I. It is interesting to note that the Clarkan sample showed up much better in quality with Formula II. With this method of test baking, all the winter-wheat samples except Cheyenne, Chiefkan, and Superhard appeared to be equal to the Renown samples in quality. The failure of Cheyenne to fall within the range of the other wheats may be due to inadequacy of the mixing. It was reported by Blish and Sandstedt (1935) that wheat of this variety re-

TABLE II

COMPARISON OF HARD RED SPRING AND HARD RED WINTER WHEAT VARIETIES, 1939
—BAKING DATA FOR FORMULA II WITH VARYING AMOUNTS OF $KBrO_3$

Variety	Loaf volume (ml) at bromate levels of:					Crumb texture at bromate levels of:				
	0	1 mg	2 mg	3 mg	4 mg	0	1 mg	2 mg	3 mg	4 mg
1 Thatcher	1015	1200	1190	1060		9.5	8.5	8.5	8.5	7.2
2 Thatcher	950	1075	1055	975	880	9.5	10.0	8.7	8.5	8.2
3 Thatcher	890	980	955	865	825	8.7	9.5	9.5	8.7	7.5
4 Thatcher	830	850	820	770	735	9	9.7	10.0	9.2	9.2
5 Thatcher	770	815	765	725	700	8.2	9.7	9.7	8.7	8.5
6 Renown	860	1040	1065	975	945	7.2	9.0	8.5	8.5	8.2
7 Renown	835	960	985	915	845	7	8.7	8.7	8.5	8.0
8 Renown	795	900	905	880	785	7	8.7	9	8.5	8.0
9 Turkey	800	860	870	850	850	6	8.5	8.7	9.2	8.5
10 Kanred	750	940	1000	960	960	5	9	9.5	9.2	8.7
11 Tenmarq	885	1000	1015	960	895	8	9.5	10.0	9.5	9
12 Blackhull	895	935	965	950	920	7.5	9.0	9.5	9.3	9
13 Superhard Blackhull	685	835	870	875	880	3.5	8.7	9.0	9	8
14 Chiefkan	615	665	650	680	662	3.5	5.5	6.7	7.5	6.5
15 Cheyenne	885	910	890	835	785	9.0	9.5	9.0	8.7	8.0
16 Nebred	985	1075	1070	1015	1005	8.7	9.5	8.5	8.5	8.5
17 Kawvale	710	750	738	725	720	5	8.2	8	7.5	7.5
18 Clarkan	785	845	865	825	820	7	8.5	8.5	8	8

quired both severe mixing and bromate. The mixing given by the Hobart mixer is hardly severe enough to develop a dough to its maximum. As a matter of fact, it is known from the recording-dough-mixer curves, that the doughs of Cheyenne are taken long before the maximum development has occurred.

Both Chiefkan and Superhard were very much below all the other varieties in quality with all baking formulas. This is in agreement with the results reported by Larmour, Working and Ofelt (1939) and others. There can be little doubt that as far as the ordinary baking technique goes, these two varieties must be regarded as inferior.

Dough-mixing curves of all these varieties were made by means of the National-Swanson micro machine. They are shown in Figure 3. No attempt will be made to associate the curve characteristic with the baking results. A casual inspection will show that very little can be expected from such a study. It can be seen, however, that there was a great deal of differentiation between the winter-wheat varieties, and some distinction between the two spring-wheat varieties. It is interesting to note that Cheyenne and Nebred, both of which gave long mixing maxima somewhat comparable to those of Thatcher, nevertheless were clearly differentiated from the latter by showing a characteristic flattening of the curve in the early stage of mixing. This feature was not found with the hard spring wheats, nor with the other winter wheats.

Tenmarq, which is a cross between Marquis and Turkey, gave a curve having the general characteristics of the Renown curves. The Thatcher samples gave the long smooth type of mixing curve thought to be typical
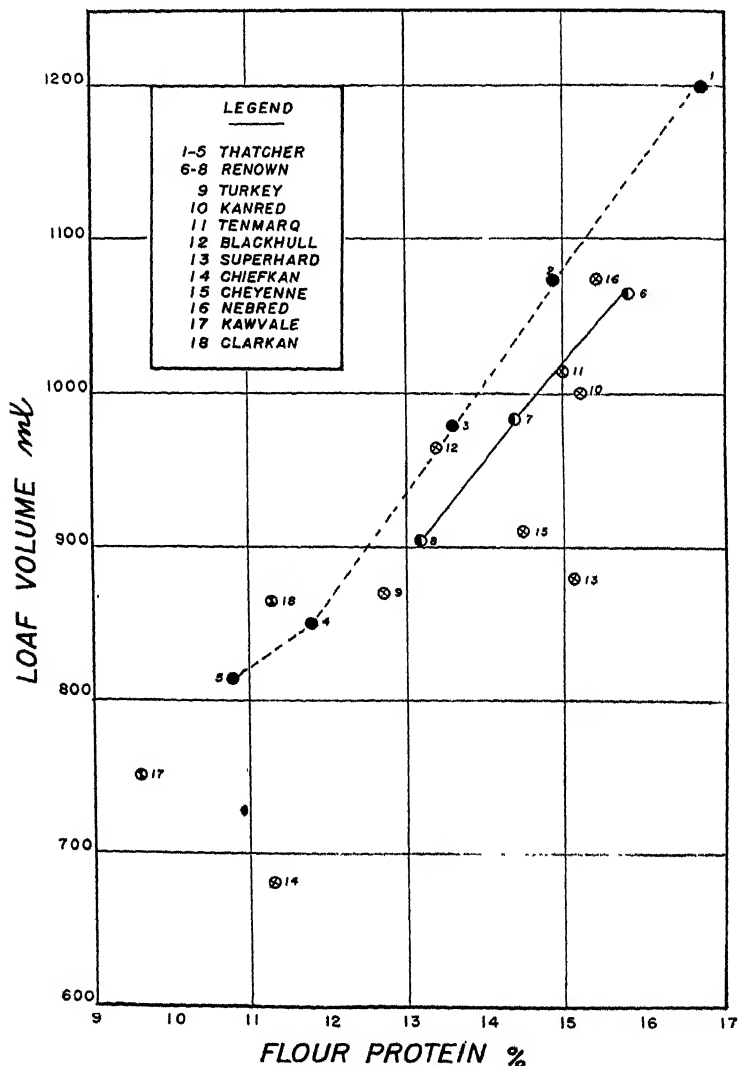
Fig. 2. Relation of loaf volume to flour protein, Formula II.

of the hard spring wheats, although it should be pointed out that as the protein content increases, there is a tendency for the curve to become sharper, a feature that was noted by Larmour, Working, and Ofelt

(1939) in connection with high-protein Turkey, Kanred, Tenmarq, and Cheyenne.

This study has added no new light on the meaning of the mixing curves. They appear to be useful as a means of differentiating varieties on the basis of mixing requirements of the flour-water doughs. There seems to be no doubt that this is a varietal characteristic, and that there is a very useful range of differences between varieties. It is therefore applicable as a means of identifying types of wheats or flours,

HARD RED SPRING

HARD RED WINTER
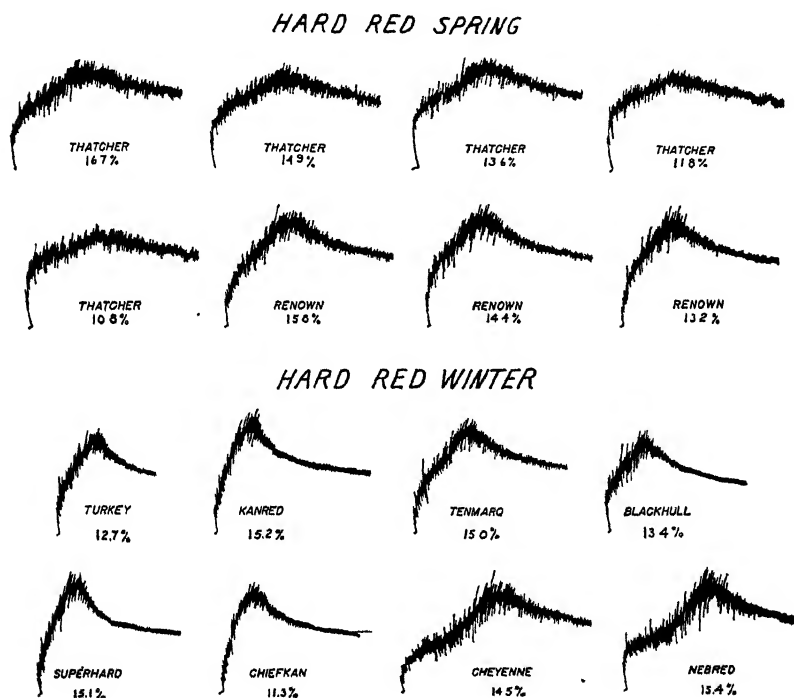
Fig. 3. Mixing curves by the National Swanson-Working recording mixer.

and for certain purposes and in certain areas this identification may be of great importance to the miller and baker, inasmuch as it enables them to choose the type they prefer.

Blending Capacity

While it has been impossible to establish any significant difference between hard red spring and hard red winter flours in loaf volume when they are baked by the ordinary methods, it has always seemed likely that the two classes of wheat might be different in their behaviour when

blended with weaker wheats or flours. It is difficult to know just what the European millers and bakers expect of a blend, and it is still more difficult to obtain the exact sorts of wheat with which the miller customarily blends the American wheats. A thorough study of blending would involve a great many kinds of weak wheat as well as many combinations of the weak and strong components of the blend. It must be realized, therefore, that the experiments reported here represent merely an approach to the problem and the data and conclusions can be regarded as only a preliminary survey.

The flour used as the weak component was a pastry flour milled from soft Ontario wheat. It had a protein content of 7.6% and baked to a volume of about 500 ml with the rich formula. The bread had a heavy, coarse, and wholly undesirable texture. Blends containing 20%, 40%, 60%, and 80% respectively of this flour were baked by Formula II, with 0.001% bromate. Careful observations were made regarding the handling properties of the various doughs in order to see if any distinction could be made on the basis of "tenderness." The baking results are given in Table III, for a number of the samples. Some of the data are shown graphically in Figure 4.

In making comparisons of blends, it is just as necessary to take the protein content into consideration as when one is comparing data obtained on ordinary samples. Consequently, in Figure 4 the loaf volume for each of the blend levels, 20%, 40%, and 60% strong flour, have been plotted against the protein of the blend. The data of each level have been designated by distinctive symbols and joined up by the straight line which seemed to fit the data best. The hard red spring wheat samples are indicated by the more heavily marked symbols. ·

It should be noted that while the volumes for each blend level have been shown separately, the relation of all the loaf volumes to protein content is as high as might be expected from random samples of one class of wheat. The correlation coefficient of loaf volume and protein content for the 20%, 40%, and 60% blends was + .94. The hard spring samples are quite well distributed within the swarm and show no evidence of being different from the hard winter samples in loaf volume of their blends. The varieties Kanred and Superhard Blackhull fall out of line in the 60% blends.

These data indicate that there may be a tendency for the lower blends to be relatively more effective than the higher blends. This is shown by the somewhat slight but fairly regular displacement of the regression curve to the right as one goes from the lower to the higher blends. If this trend is real, it also indicates that higher-protein wheats are more effective in blends than low-protein wheats, even when the

TABLE III
BAKING DATA FOR BLENDS WITH ONTARIO WINTER WHEAT PASTRY FLOUR

Sample No.	Description of sample	Percent of strong flour	Flour protein	Loaf volume	Crumb texture
		%	%	ml	score
6	Renown	20	9.3	598	3.5
		40	10.9	751	8
		60	12.5	884	9
		80	14.1	950	10
3	Thatcher	20	8.8	558	3.5
		40	10	650	6
		60	11.2	754	8.5
		80	12.3	875	10
16	Nebred	20	9.2	627	4
		40	10.8	779	8
		60	12.2	885	9.5
		80	13.8	978	10
12	Blackhull	20	8.8	602	3
		40	10.0	655	5
		60	11.0	709	7
		80	12.2	833	9
9	Turkey	20	8.6	557	3
		40	9.7	633	5
		60	10.6	694	7.5
		80	11.7	774	8
10	Kanred	20	9.1	598	3
		40	10.7	702	5.5
		60	12.1	765	7.5
		80	13.7	845	8
11	Tenmarq	20	9.1	605	5.5
		40	10.6	715	8
		60	12.0	820	9.5
		80	13.5	888	10
13	Superhard	20	9.1	605	3.5
		40	10.6	671	5.5
		60	12.1	715	6.5
		80	13.6	750	7
14	Chiefkan	20	8.4	546	1
		40	9.2	594	3
		60	9.8	612	3.5
		80	10.5	614	3.5
15	Cheyenne	20	9.0	602	5.5
		40	10.4	700	7.5
		60	11.7	793	10
		80	13.1	851	10

protein content is taken into consideration. Thus a high-protein flour that yields a blend of 10.9% protein in a 40% blend gives about 35 ml larger loaf volume than a lower-protein sample which in a 60% blend yields a protein content of the same value. This deduction is quite opposite to that of Sandstedt and Ofelt (1940), who in a study of flour-starch blends reported indications of a retrogradation of protein quality with increasing protein content.
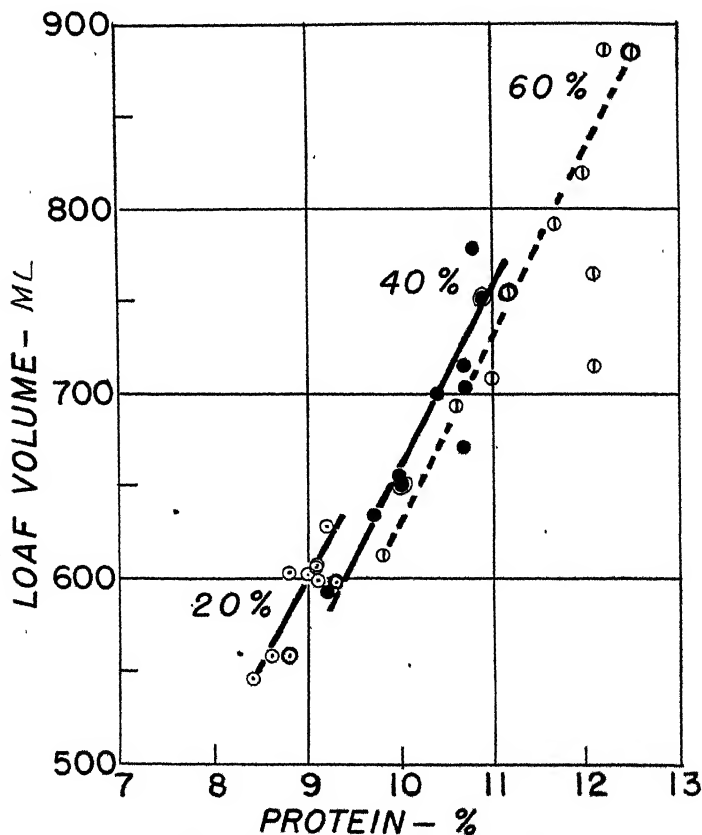
Fig. 4. Relation of loaf volume to flour protein, blends with Ontario soft winter flour.

With regard to handling properties of the doughs, the baker was convinced that there was considerable difference between the hard winter flours and the hard spring flours except in the cases of Nebred and Cheyenne, which he reported behaved like the spring wheat flours. As the descriptive terms used to differentiate the various doughs would have no meaning except for the individual applying them, it was not consid-

ered of any value to include them in this report. The principal criticism of the majority of the hard winter samples was that they produced "tender" doughs. By this was meant that they gave up their gas more readily than the hard spring flours, and showed less tendency to become bucky on handling.

It seems probable that the quality described as "tenderness" might show up better in hearth than in tinned loaves. The softer doughs might be expected to flow out in proofing and give a less bold loaf than the tougher spring wheat flour blends. This possibility was not examined in this study on account of shortage of the supply of the hard red winter flours.

Conclusions

The data presented in this study, although based on a small number of samples chosen at random, seem to support the author's earlier conclusions (1940) based on the published data of numerous workers, that there is no conclusive evidence that the hard red winter wheats are inferior to the hard red spring wheats in intrinsic baking quality, as tested by experimental methods available at the present time.

Tests made with soft wheat flour blends indicated that as far as loaf volume and crumb texture were concerned, there was no distinction between flours of the hard winter and hard spring classes. There may have been a qualitative difference in the handling properties of the doughs, but it was a distinction too elusive to be described with any degree of accuracy.

Dough-mixing curves obtained with the National-Swanson recording mixer show marked differences in the mixing characteristics of varieties of both classes of wheat, but none of the differences recorded seem to be directly related to the baking performance of the flours.

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AN APPARATUS FOR THE MEASUREMENT OF BREAD CRUMB DEFORMATION

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Measurements of the elastic and plastic deformation of bread crumb resulting from the application of weight and pressure are of great interest from both the theoretical and the practical point of view. Indeed a thorough study of dough maturation, bread baking, the evaluation of bread crumb quality, the process of cooling, and storage of kinds of bread is quite impossible without a determination of these properties. It is, however, true that insufficient consideration has hitherto been given to this problem. The reports of Katz (1928), of Platt and Powers (1940), and of some other investigators who studied the bread staling process by observing the changes occurring in the compressibility (total deformation) of bread crumb during the storage period are well known.

In the U.S.S.R. work along these lines was conducted at the All Union Institute for Bread Baking Research (by Kuhlman-Balashova). Bakers have habitually noted the fact that on application of pressure with a finger poorly baked bread crumb will but slightly recover its initial volume, while well baked crumb possesses a better capacity for partial recovery after general deformation. That is why in the standards for baked bread adopted in the U.S.S.R. the following specification for the organoleptic (by feeling) characteristic of crumb is to be found: "Upon application of light pressure on the crumb with a finger, it must quickly recover its original form."

It was while making use of this practice of bread penalization, that Axelrode suggested a special device for determining whether bread is thoroughly baked. This device permitted measurements of the elastic deformation of its crumb.

While studying in 1938–39 the properties of elastic and plastic deformation of bread crumb, the author tested a number of devices employed for this purpose and recognized the need for an improved and more precise measuring apparatus. Since then such an apparatus has been constructed by the Technological Laboratory of the Institute under his supervision. The present paper aims to describe and discuss the new improved apparatus for measurements of elastic and plastic deformation, and the method of its application.

Description of the Apparatus

The structural principle of the apparatus is essentially the same as that of Axelrode but with the following additions and changes:

1. The apparatus is not only suitable for measurements of elastic deformation properties under an unlimited load according to Axelrode's method, but is also applicable to measurements of the total deformation (compressibility) of the crumb.

2. The principle of the action of an unlimited load has been replaced by the action of a definite load within a stated period of time.

3. The sensitivity of the apparatus has been increased approximately 2.5 times that obtainable with the device of Axelrode. This has been done at the expense of increasing the ratio of lever arms— "pressing finger"—and lever measuring pointer.
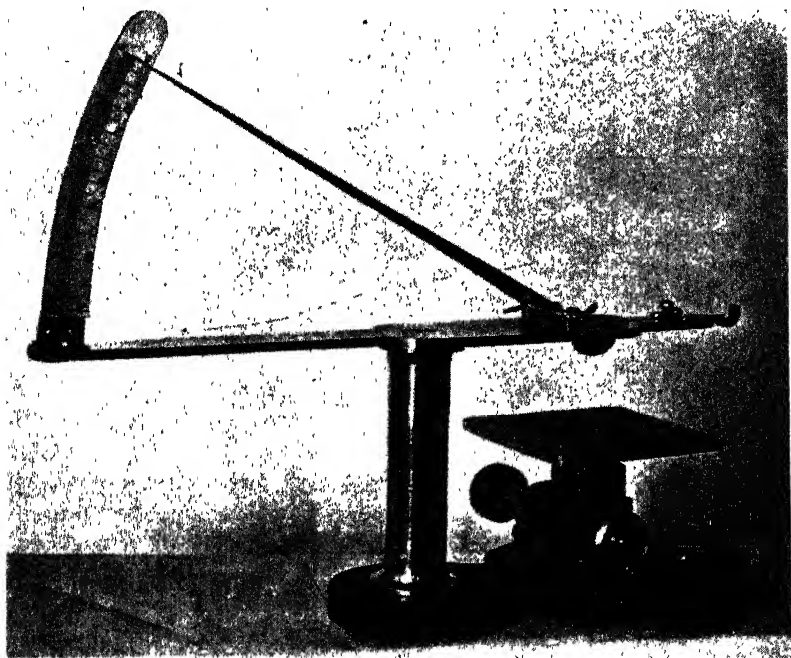
Fig. 1. Apparatus for the measurement of deformation of bread crumb.

4. In contradistinction to the device of Axelrode, which is set directly on bread, the apparatus described is constructed on the principle of the action of only the working member upon an accurately sliced piece of bread crumb of a determined dimension. The apparatus consists of a flat metallic base (A) (Fig. 2) with a support (B) mounted on it, to which a second flat metallic foundation (C) is attached. A

pointer-lever (D) and a measuring scale (E) are mounted on the foundation (C).

The lever is balanced in such a way that there is a light overweight in the shorter arm ending in a semispherical "finger" (F) to which the hook (G), to hang the weight on, is attached. Consequently the right lever arm is in horizontal position, while the semispherical surface overlaps through the opening by 10 mm beyond the lower base of the apparatus.
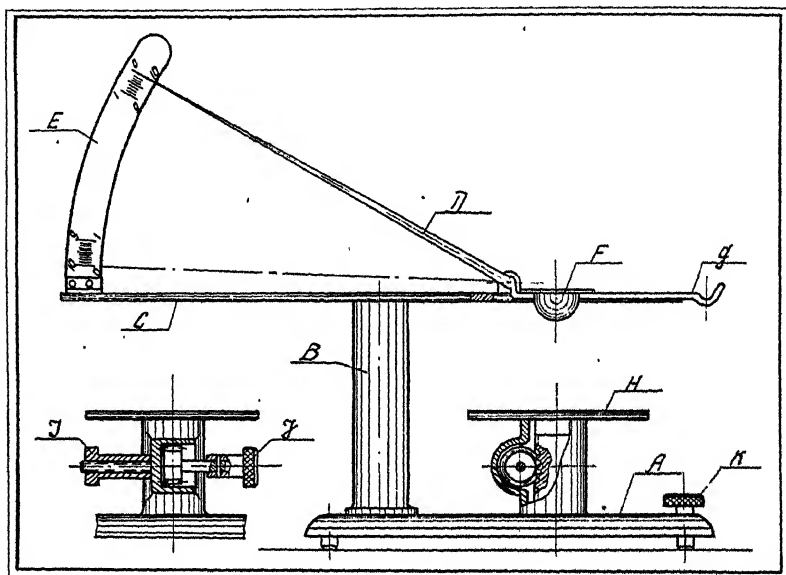
Fig. 2. Detailed drawing of the apparatus.

The distance from the center of the finger semisphere to the point of support is one-tenth of the distance from the support to the end of the measuring pointer.

The left longer lever arm (measuring pointer) usually points to the figure 10 (on the left-hand side of the scale).

Above the lower base of the apparatus (opposite the opening) a lifting platform (H) is to be found which with the help of special screws (lifting and stop-screws) may be raised and fixed at the requisite height.

Operation of the Apparatus

The loaf to be tested is divided into two equal parts; then from each half, on the cut side, a slice of 5×5 cm and 4 cm thick is cut. In order to reduce errors due to uneven porosity, each loaf is subjected to two

parallel determinations along the central part of the cut. The cut is made with a sharp knife, and the resulting crumbs are carefully removed from the surface of the loaf.

The slice of bread to be tested is placed on the lifting platform, being located in such a way that the central part of the cut is exactly under the opening in the base of the apparatus (in case of irregular porosity in the center, the cut must be slightly shifted). Now with a special screw (*I*) the platform is raised into such a position that the lower surface of the base makes close contact with the upper surface of the cut, the indicating pointer being at zero. Thereupon the platform is fixed in the necessary position by means of the second screw (*J*).

A beaker of waxed paper must be hung on the hook and a measured quantity of water introduced through a pipette into it. The quantity of water depends on the kind of bread being tested and the degree of its staleness. Simultaneously a sand-glass is tilted.

After the expiration of one minute the position of the pointer on the measuring scale is recorded. The position shows the total deformation (compressibility) of the bread crumb. In order to measure the elastic deformation (elasticity) of the crumb, the weight is removed after one minute of load application (the semispherical part of the apparatus touches at that moment the bread surface) thus releasing the pointer, which is left in this position for two minutes more. Under the action of the elastic properties of bread, the "finger" of the apparatus is raised upwards and the indicating pointer goes down.

The difference in the readings taken at the position of the pointer under load and its position two minutes after the removal of the weight characterizes the elastic deformation of the crumb in terms of the scale units. Relative elasticity is calculated as the percentage—ratio of the elastic deformation to total deformation, the latter being taken as 100%.

The difference between duplicate measurements of general compressibility should not exceed 0.5 of the scale units of the apparatus, otherwise a third measurement must be made. Two concordant readings are considered to show compressibility.

Possibilities for Utilization of the Apparatus

Preliminary data on the work conducted by the Technological Laboratory of our Institute with the described apparatus as well as with other devices for measuring bread crumb deformation permit us to outline the possible field of practical utilization of the apparatus and the methods of measurement.

1. Measurements of the bread crumb deformation during heating in the baking process show that well heated (well baked) wheat bread

made of straight flour possesses a definite degree of relative elasticity, which usually is not less than 80%. The rate of compressibility declines as heating progresses, finally reaching a limit. Consequently the degree of maturity of bread or the period of time necessary for baking may be established by an objective method which records the magnitude and the dynamics of relative elasticity, as well as the compressibility of the crumb. These measurements provide possibilities for outlining the more efficient and rational methods of baking and for indicating the economical utilization of ovens.
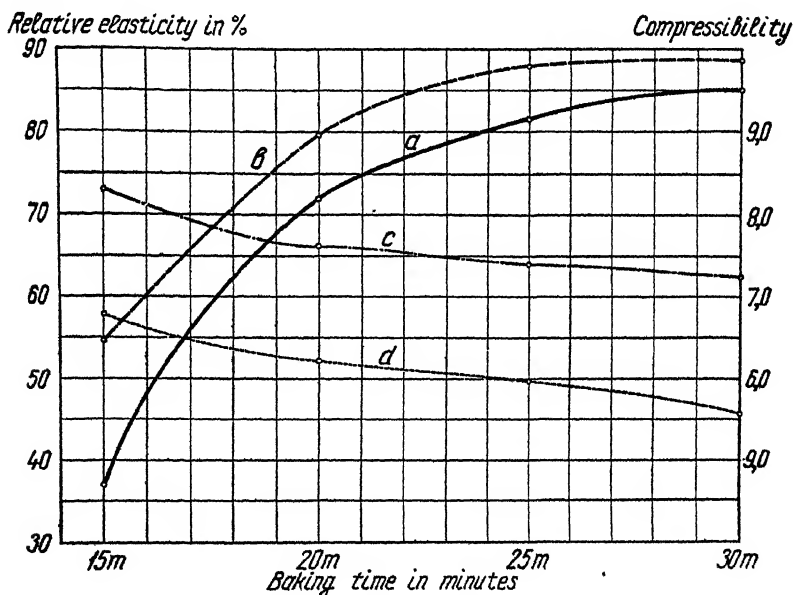
Fig. 3. Changes in the compressibility-elasticity properties of bread crumb—baking time 15–30 minutes: a, elasticity 1 hour after baking; b, elasticity 6 hours after baking; c, compressibility 1 hour after baking; d, compressibility 6 hours after baking.

Figure 3 shows values for total and elastic deformation as measured with the apparatus, using straight-flour pan bread. The experiment was conducted in the following way: from the same dough four breads were simultaneously baked with different oven periods (15, 20, 25, and 30 minutes). After one hour of cooling the loaves were cut into two parts; from one-half of each loaf measurements were made of total and elastic deformation and from the second half the same measurements were made after four hours of cooling. The results of the measurements were taken as a basis for two curves, showing decrease of total deformation (compressibility) and increase of elastic deformation as related to length of baking period.

Of great interest is the fact that the elastic properties of insufficiently baked bread showed an improvement upon additional standing after baking. Bread baked in an oven for 15 and 20 minutes showed considerable difference in the magnitude of elastic deformation after one and after four hours of standing. However, even after four hours of standing the total magnitude of elastic deformation was still very low if compared with bread baked during 25–30 minutes. It is also to be noted that the angle of inclination of the curve showing changes in elastic deformation decreased in proportion to the increase of baking period. It shows that after a certain time of baking, the bread reaches a certain practically constant magnitude of elastic deformation, which apparently characterizes the end of the baking period.

2. Insufficiently baked or "doubtful" bread is sometimes left to "season." After a certain lapse of time the bread is again tested for maturity according to the elasticity of the crumb. Preliminary measurements have shown that our apparatus reliably records changes in the elastic properties of bread in the processes of its cooling and storage. Therefore this apparatus enables one to establish the time of seasoning, after which the bread may either be delivered for sale or penalized.

3. Measurements of total deformation provide numerical expression for the quality and the condition of bread crumb and grain. The measurements show that bread crumb with small, well developed thin-wall grain gives a higher magnitude of total deformation (compressibility). If bread is thoroughly baked it will also have high elasticity. Bread with a coarse thick-wall "hornlike" grain shows a low magnitude of total deformation although it may possess high relative elasticity. Consequently when evaluating the "quality" of grain in a bread of a definite grade not only the magnitude of elasticity, but also the compressibility should be duly taken into account.

The magnitude of compressibility and elasticity thus completes an evaluation of the bread baking qualities of selected wheats. Preliminary measurements with this apparatus conducted in the Laboratory for Bread Testing of our Institute (Losev, Zavyalova) have shown that wheats with high baking qualities scored high with respect to total and elastic deformation, while wheats with medium baking qualities showed much lower total deformation and somewhat lower elastic deformation.

4. Investigations conducted by the Technological Laboratory of the Bread Baking Institute seeking to obtain more precise data on minimum seasoning periods for rye-wheat bread prior to cutting it for drying into biscuits proved that this time is also determined by the magnitude of total and elastic deformation. A bread having too large

total deformation (compressibility) and at the same time a low elastic deformation, will be pressed down. For low compressibility (very stale bread) the texture of bread grain seems to be impaired and the biscuits will have small branchlike cracks over the whole of their surface.

5. Finally, the studies of the Technological Laboratory of the Institute have shown that compared with other physicochemical methods the ascertainment of total deformation of the bread crumb in the course of its storage affords the best measurement of the staling process.

The Technological Laboratory of our Institute is continuing the work with a view toward further improvement in precision of the methods for measuring bread crumb deformation.

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THIAMIN CONTENT OF COMMERCIAL WHEATS OF THE 1940 CROP

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It has been pointed out by Schultz, Atkin, and Frey,[2] that there is a considerable range in the level of thiamin in various wheat samples. This study was undertaken to secure further data on the variation that may be expected from wheats of different varieties or from different localities. Because the miller seldom grinds a lot of wheat consisting of a single variety, it was thought that the use of commercial samples would afford a useful basis for making comparisons. Thus most of the wheats analyzed were commercial samples.

Assays were made by the fermentation method of Shultz, Atkin, and Frey.[3] The samples were ground in a Quaker City mill set at the

TABLE I

SOURCES, GRADES, AND THIAMIN VALUES OF WHEAT SAMPLES

Lab. No.	Origin—state, county or locality	Grade and market class	Variety	Test weight	Remarks	Thiamin
				lbs		*μg/g*
W-9	Colo., Denver	2 Dark hard winter	—	—	—	8.0
W-14	Colo., Denver	2 Dark hard winter	—	58.8	—	8.4
W-29	Colo., Denver	2 Dark hard winter	—	—	—	7.7
W-90	Eastern Colo. farm	1 Dark hard winter	—	60.0	—	6.7
W-11	Dakota [1]	3 Dark northern spring	Thatcher	—	—	5.3
W-38	Dakota [1]	2 Dark northern spring	Thatcher	—	—	6.6
W-44	Dakota [1]	2 Dark northern spring	Thatcher	—	—	7.8
W-46	Dakota [1]	1 Dark northern spring	—	—	—	7.7
W-51	Dakota [1]	2 Dark northern spring	—	—	—	7.9
W-57	Dakota [1]	2 Dark northern spring	—	—	—	5.9
W-54	Dakota [1]	3 Hard white	Burbank	—	—	7.5
W-60	Dakota [1]	2 Dark northern spring	—	—	—	5.1
W-24	Dakota [1]	1 Red durum	—	—	—	6.7
W-25	Dakota [1]	2 Hard amber durum	—	—	—	6.4
W-28	Dakota [1]	2 Hard amber durum	—	—	—	6.5
W-31	Dakota [1]	2 Hard amber durum	—	—	—	7.5
W-34	Dakota [1]	4 Hard amber durum	—	—	—	7.7
W-36	Dakota [1]	2 Hard amber durum	—	—	—	6.9
W-40	Dakota [1]	3 Hard amber durum	—	—	—	7.7
W-50	Dakota [1]	2 Red durum	—	—	—	8.0
W-52	Dakota [1]	4 Red durum	—	—	—	8.2
W-41	Dakota [1]	1 Heavy northern spring	—	—	—	5.4
W-66	Idaho, Newdale	1 Dark hard winter	—	62.1	84% dark hard winter	6.4
W-2	Illinois, Pratt County	2 Dark hard winter	—	—	—	5.2
W-16	Illinois, Central	2 Hard winter	—	—	—	5.9
W-45	Illinois, Central	2 Soft red winter	—	—	—	5.9
W-55	Illinois, Central	2 Hard winter	—	—	—	6.9
W-53	Indiana	- Soft red winter	—	—	—	8.0
W-92	Iowa, Council Bluffs	1 Hard winter	—	—	—	7.6
W-85	Kansas, Salina	2 Dark hard winter	Tenmarq	59.6	—	7.2
W-101	Kansas, Gueda Springs	2 hard winter	Tenmarq	59.8	—	6.0
W-105	Kansas, Silver Lake	1 Dark hard winter	Tenmarq and Iowa Red	62.1	14.20% protein	7.4
W-106	Kansas, Clay Co.	1 Hard winter	Tenmarq	60.5	13.35% protein	7.8
W-107	Kansas, Riley Co.	1 Hard winter	Tenmarq	61.1	11.65% protein	6.8
W-108	Kansas, Geary Co.	1 Hard winter	Tenmarq	61.0	12.25% protein	8.2
W-113	Kansas, Riley Co.	1 Hard winter	Tenmarq	61.1	12.50% protein	6.2
W-115	Kansas, Washington Co.	2 Hard winter	Tenmarq	59.2	8% Kawvale	7.7
W-109	Kansas Dickinson County	1 Hard winter	Tenmarq and Blackhull	60.0	12.85% protein	6.4
W-110	Kansas, Russell Co.	1 Dark hard winter	Blackhull	61.1	15.20% protein	7.3
W-116	Kansas, Republic County	1 Dark hard winter	Tenmarq and Blackhull	60.4	13.80% protein	8.0
W-82	Kansas, N. Central	1 Hard winter	Blackhull	63.0	—	6.1
W-114	Kansas, Marion County	2 Hard winter	Chiefkan, Turkey, Tenmarq	58.4	11.85% protein	6.4
W-112	Kansas, Ellsworth County	Sample—Dark hard winter	Turkey and Blackhull	55.6	1% dock, musty odor, 14.55% protein	7.8
W-84	Kansas, N. Central	1 Dark hard winter	Turkey	60.8	—	7.4
W-83	Kansas, N. Central	1 Hard winter	Redhull	62.4	—	5.8
W-4	Kansas	2 Dark hard winter	—	58.0	—	5.1
W-5	Kansas, Wichita	2 Dark hard winter	—	58.1	1.0 dock	6.1
W-10	Kansas	1 Hard winter	—	60.4	—	5.0
W-15	Kansas	2 Dark hard winter	—	58.0	—	6.2
W-21	Kansas Wichita	4 Dark hard winter	—	55.0	—	6.4
W-27	Kansas	1 Hard winter	—	60.0	—	7.8
W-35	Kansas	3 Red winter	—	58.8	8% shrunken and broken	6.4
W-37	Kansas, Wichita	2 Dark hard winter	—	58.1	—	7.8
W-80	Kansas, Salina	2 Dark hard winter	—	59.5	Med. high protein terminal elevator	8.7

TABLE I—*Continued*

Lab. No.	Origin—state, county or locality	Grade and market class	Variety	Test weight	Remarks	Thia-min
				lbs		*µg/g*
W-81	Kansas, Salina	2 Dark hard winter	—	58.5	Low protein terminal elevator wheat	6.6
W-88	Kansas, Home	1 Hard winter	—	61 4	—	10.2
W-102	Kansas, Arkansas City	2 Hard winter	—	59.0	—	6.3
W-117	Kansas, Seward Co.	2 Dark hard winter	—	59.8	—	7.6
W-118	Kansas, Seward Co.	1 Dark hard winter	—	60.5	—	8.8
W-119	Kansas, Clay Co.	1 Hard winter	—	60.3	—	6.6
W-120	Kansas, Morris County	2 Hard winter	—	59 3	—	8.2
W-121	Kansas, Morris County	2 Hard winter	—	59.6	—	7.9
W-122	Kansas, Dickinson County	2 Hard winter	—	58.0	—	4 6
W-123	Kansas, Shawnee County	1 Hard winter	—	61.1	Protein 12.06, 15% moisture	8.6
W-124	Kansas, Clay Co.	1 Hard winter	—	60.5	Protein 12.81, 15% moisture	6.0
W-126	Kansas, Riley Co.	1 Hard winter	—	61.1	Protein 11.52, 15% moisture	7.4
W-127	Kansas, Riley Co.	1 Hard winter	—	61.1	Protein 12.09, 15% moisture	7.7
W-128	Kansas, McPherson Co.	2 Hard winter	—	59.5	Protein 12.12, 15% moisture	6.9
W-129	Kansas, Shawnee County	1 Dark hard winter	—	62.1	Protein 13.49, 15% moisture	8.0
W-130	Kansas, Geary Co.	1 Hard winter	—	61.0	Protein 11.85, 15% moisture	8.5
W-131	Kansas, Dickinson County	2 Dark hard winter	—	59.5	Protein 13.10, 15% moisture	9.5
W-132	Kansas, Lane Co.	2 Dark hard winter	—	59.3	Protein 15.49, 15% moisture	9.9
W-133	Kansas, Dickinson County	2 Hard winter	—	59.5	Protein 12.46, 15% moisture	8.7
W-94	Kansas, Doniphan County	1 Soft red winter	Harvest Queen	60.5	—	5.8
W-114	Kansas, Shawnee County	2 Mixed—75% hard 25% soft	Tenmarq, Iowa Red (hard) Kawvale (soft)	59.5	—	6.9
W-125	Kansas, Shawnee County	1 Mixed—80% hard 20% soft		60.0	Protein 12.37, 15% moisture	5.5
W-62	Maryland	2 Soft red winter	—	—	Garlicky	6.6
W-20	Minnesota	3 Hard winter	Mintuski	—	—	7.2
W-48	Missouri	3 Soft red winter	—	—	—	5.3
W-58	Missouri	– Soft red winter	—	—	—	7.3
W-95	Missouri, Buchanan Co.	1 Red winter	Clarkan	62.0	—	6.3
W-96	Missouri, Buchanan Co.	2 Red winter	Kawvale	58.7	—	5.3
W-97	Missouri, East Central	1 Red winter	Buttercup	60.2	—	6.0
W-98	Missouri, Stewartsville	1 Hard winter	(Turkey Red?)	61.3	"Typical Mo. Yellowberry"	4.1
W-43	Montana	2 Northern spring	—	—	—	6.5
W-63	Montana	1 Dark northern spring	—	—	—	7.0
W-3	Nebraska, Western	3 Dark hard winter	—	—	—	6.6
W-13	Nebraska, Eastern	1 Hard winter	—	—	—	6.9
W-22	Nebraska, Eastern	1 Hard winter	—	—	—	7.3
W-23	Nebraska, Eastern	2 Hard winter	—	—	—	6.5
W-89	Nebraska, Eastern	1 Dark hard winter	—	61.8	—	7 4
W-91	Nebraska, Eastern	3 Hard winter	—	57.2	—	9.4
W-93	Nebraska, Eastern	2 Hard winter	—	59.0	—	9.2
W-134	Nebraska, Plymouth	2 Dark hard winter	—	58.8	1% damaged	7.8
W-39	Ohio	– Soft red winter	—	—	—	5.8
W-42	Ohio	– Soft red winter	—	—	—	6.6
W-47	Ohio	– Soft red winter	—	—	—	6.8
W-49	Ohio	– Soft red winter	—	—	—	6.1
W-59	Ohio, Eastern	3 Soft red winter	—	59.0	4.8% damaged	5.9
W-61	Ohio, Western	2 Soft red winter	—	59.5	2.5% damaged	5.1
W-56	Ohio, East Central	1 Soft red winter	—	60.0	1.9% dockage	6.6
W-6	Oklahoma	1 Dark hard winter	—	60.4	—	6.2
W-7	Oklahoma	2 Hard winter	—	—	—	5.4
W-8	Oklahoma	2 Hard winter	—	60.7	3.2% damaged	5.9
W-19	Oklahoma	2 Hard winter	—	59.0	—	6.0
W-26	Oklahoma	2 Hard winter	—	59.2	—	5.1
W-30	Oklahoma	1 Dark hard winter	—	61.3	—	8.2
W-32	Oklahoma	1 Hard winter	—	60.4	—	5.1
W-99	Oklahoma	2 Hard winter	—	60.0	10% soft wheat	7.8
W-100	Oklahoma	2 Hard winter	—	58.7	—	7.2
W-103	Oklahoma	1 Mixed red winter 88% hard 12% soft	—	60.2	—	5.8
W-104	Oklahoma	2 Mixed—75% hard 25% soft	—	59.5		5.1

TABLE I—*Continued*

Lab. No.	Origin—state, county or locality	Grade and market class	Variety	Test weight	Remarks	Thia-min
				lbs.		*μg/g*
W–68	Oregon, Klondike	4 Hard white	—	55.0	—	4.6
W–75	Oregon, Biggs	3 Hard white	—	57.0	—	6.0
W–77	Oregon, Miller	3 Hard white	—	57.7	—	6.0
W–76	Oregon, The Dalles	2 soft white	—	58.6	—	7.0
W–1	Texas	1 Dark hard winter	—	60.8	1.9% dockage	5.8
W–12	Texas	2 Dark hard winter	—	60.6	3 8% dockage	7.0
W–17	Texas	1 Dark hard winter	—	60.5	—	5.2
W–33	Texas	2 Dark hard winter	—	59.7	—	7.4
W–86	Texas, Cook Co.	2 Mixed red winter	Mediterranean red	58.0	Not true to variety	6.9
W–87	Texas, Hale Co.	1 Dark hard winter	Turkey	61.5	—	8.1
W–135	Texas	1 Dark hard winter	—	60.6	1% dockage	6.7
W–136	Texas	1 Dark hard winter	—	60.8	1% dockage elevator wheat	8.0
W–137	Texas	1 Dark hard winter	—	61.6	Elevator wheat	7 6
W–138	Texas	1 Dark hard winter	—	60.5	Elevator wheat	8 1
W–139	Texas	1 Dark hard winter	—	61.5	—	7.5
W–140	Texas	1 Dark hard winter	—	61.8	—	7.8
W–141	Texas	1 Dark hard winter	—	61.7	—	7.5
W–142	Texas	1 Dark hard winter	—	60.7	—	4.2
W–143	Texas	1 Dark hard winter	—	61.7	—	6.6
W–67	Utah, Garland	1 Hard winter	—	61.2	—	4.1
W–64	Washington, Waitzburg	3 Soft white	—	56.0	—	4.2
W–69	Washington, Cowship	1 Soft white	—	60.0	—	4.6
W–78	Washington, Hadley	1 Soft white	—	60.5	—	7.4
W–65	Washington, Coulee City	3 Hard white	—	57.8	—	7.1
W–70	Washington, Benge	1 Hard white	—	60.2	—	4.5
W–71	Washington, La Crosse	1 Hard wnite	—	61.0	—	6.0
W–72	Washington, Waitzburg	1 Western red	—	61.5	—	6 8
W–79	Washington, Cashub	1 Western red	—	60.5	—	7.0
W–73	Washington, Garfield	1 White club	—	60.5	—	7.0
W–74	Washington, Dayton	3 White club	—	56.5	—	5.6

[1] Includes both North Dakota and South Dakota.

finest grinding position. After being ground the samples were held in sealed jars in a refrigerator until assayed. All data are given on an " as received " basis, as it was found that conversion to a 15% moisture basis did not change the results other than to lower all values by approximately 0.4 μg. Since the samples were secured during the early part of 1941 it may be assumed that they probably are all from the 1940 crop. There was no correction for a sulfite blank, since it was found that such a correction would be so small as to be within the experimental error.

One hundred and forty-nine samples of wheats with histories indicating origin in at least 18 states, were obtained. Classified by market types the samples include 85 hard red winter, 15 hard spring, 17 soft red winter, 12 white wheats, 9 durum wheats, 9 mixed red winter (predominantly hard), and 2 western red wheats. The thiamin values and detailed data are presented in Table I.

Discussion

A classification of the wheats according to market types, as in Table II, shows that the " hard " wheats—hard winter, spring, and durum—

averaged 7.1 μg of thiamin per gram. The " soft " wheats—soft winter and white and club wheats from the Pacific northwest—averaged 6.1 μg per gram. This might indicate that climates that produce hard wheats with higher protein content also produce higher thiamin content.

TABLE II

THIAMIN VALUES ACCORDING TO MARKET TYPES

Type of wheat	Number samples included	Average thiamin
		μg/g
Durum	9	7.3
Spring	10	6.5
Soft winter	17	6.2
White wheat, including "club"	13	6.0
Western red	2	6.9
Hard winter	70	7.2

Table I shows more samples from Kansas than any other state, and in most instances the county of origin is shown. A line drawn across the state running north and south and passing through Manhattan will roughly show the division of types for this state. In the eastern third, soft or " mixed " wheats are produced, while west of the line the hard winter wheat is more likely to predominate. Neglecting those samples for which no county was given, wheats from the eastern part of the state averaged 7.0 μg per gram, while those west of the line averaged 7.65 μg per gram. The latter approximates the values for the Colorado samples. The average for all samples from Kansas was 7.4 μg per gram.

There was not a sufficient number of pure-variety samples grown

TABLE III

THIAMIN VALUES BY STATES

State	Type	Number of samples	Average thiamin
			μg/g
Colorado	Hard winter	4	7.7
Dakotas	Spring	8	6.5
	Durum	9	7.3
Illinois	—	4	6.0
Kansas	Tenmarq (all hard winter)	10	7.5
	All (all hard winter)	42	7.4
Nebraska	Hard winter	8	7.6
Missouri	Soft winter	6	5.7
Ohio	Soft winter	7	6.1
Oklahoma	Hard winter	11	6.1
Texas	Hard winter	15	6.7
Washington and Oregon	White	14	6.0

over a wide area to show any varietal trends. Tenmarq and Turkey samples were very near the average for the state in which grown.

Average thiamin values for the midwestern states are given in Table III, where it appears that the states producing hard winter wheat (with the exception of Oklahoma) gave thiamin values of $7\,\mu g$ per gram or more. The states producing soft red winter or white wheats had thiamin values of 5.7 to 6.1 μg. The 8 samples of Dakota spring wheat had an intermediate value of 6.5 μg. There was a wide variation among samples from any state. The values found ranged from the lowest reported by Schultz, Atkin, and Frey (1941) to 10 μg per gram, which is higher than any reported.

Summary and Conclusions

While no categorial statements about the 1940 American wheat crop can properly be based upon the number of samples assayed in this project, what may be a significant trend is noted. A higher thiamin content is found generally in the types of wheat having a hard vitreous berry, with an accompanying higher protein content than is found in the softer types. Hard wheats averaged 7.1 μg per gram as against 6.1 for the soft wheats.

THE THIAMIN CONTENT OF CEREAL GRAINS [1]

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(Read at the Annual Meeting, May 1941)

With the increasing recognition of the importance of cereals as dietary sources of thiamin has come the need for a more thorough knowledge of the natural vitamin B_1 content of whole grains. Schultz, Atkin, and Frey (1941) have made valuable contributions by their survey of American cereals and Booth (1940) in England has reported on samples of wheat which were grown in all parts of the world. The latter studies in particular have demonstrated the wide variations which occur.

During the course of our investigations a large number of samples of cereal grains have been available. These included many types and varieties grown in different sections of the country. In some instances they represented several series of the same varieties differing only in the location where they were grown. A study of these has been of particular interest since it suggests the existence of certain factors which influence the vitamin content and account for some of the variations observed.

The Hennessy and Cerecedo modification of the thiochrome method was employed for the analyses and the values recorded represent the average of duplicate determinations which agreed within 5%.

Spring Wheats

Table I gives the results obtained on six varieties of spring wheats grown at four stations in Minnesota. The average for these 24 samples was 2.75 mg per pound. In the individual samples, however, there was a considerable difference, since the Marquis grown at Crookston contained only 2 mg, while the Pilot grown at St. Paul had nearly 3.5 mg per pound or 75% more thiamin. When the averages of the different varieties grown at the same locations are considered it is apparent that the vitamin content varies with the location.

In this series of spring wheats the samples from the St. Paul station had the highest values and those from Crookston the lowest. Those from Morris and Waseca occupied intermediate positions. On the other hand, the averages for single varieties grown at the different locations did not show material differences. With the exception of Thatcher all are within practically 5% of the average.

These observations indicate that for these particular samples, at least,

TABLE I

THE THIAMIN CONTENT OF SPRING WHEATS
(Thiamin in milligrams per pound—13.5% moisture basis)

Variety	Location grown (Minnesota)				Average
	St. Paul	Morris	Waseca	Crookston	
Renown	3.12	3.15	2.61	2.32	2.80
Rival	3.15	2.85	2.76	2.41	2.80
Pilot	3.49	2.75	2.94	2.42	2.90
Thatcher	3.08	2.63	2.39	2.10	2.55
Ceres	3.28	3.18	2.68	2.17	2.83
Marquis	2.82	2.96	2.71	2.01	2.62
Average	3.16	2.92	2.68	2.24	2.75

environmental factors play a large role in deciding the thiamin content of wheat.   Varietal factors are of much less consequence.

Table II gives the protein values of these same wheat samples.   Any trends in protein did not seem to follow those found for the vitamin.

TABLE II

THE PROTEIN CONTENT OF SPRING WHEATS
(Protein in percent—13.5% moisture basis)

Variety	Location grown (Minnesota)				Average
	St. Paul	Morris	Waseca	Crookston	
Renown	16.9	16.6	15.7	15.7	16.22
Rival	15.4	14.8	14.4	14.8	14.85
Pilot	15.5	14.1	14.4	15.3	14.82
Thatcher	15.8	16.2	15.1	16.1	15.80
Ceres	15.8	15.4	14.1	15.6	15.22
Marquis	15.8	15.8	13.6	15.6	15.20
Average	15.87	15.48	14.55	15.52	15.35

Thus at Crookston where the samples were lowest in thiamin the average protein was similar to that of the Morris-grown samples.   Similarly, with the averages from the single varieties no relation to thiamin appears.
    Table III shows the values for ash.   There was a definite relationship between this constituent and thiamin, since the samples from locations where the ash contents were highest and lowest were also similarly related in vitamin content.
    These data have been analyzed statistically.   There is no significant relation between thiamin and protein, the correlation coefficient being + .127.   With ash, however, the correlation is highly significant.   Figure 1 shows this relation.   The correlation coefficient is + .680 and the 1% point is + .515 for the 24 pairs.   This means that the chances are

The ash values given in Table V are the highest for the samples grown at Dodge City. The samples were also high in thiamin. On the other hand, the Lincoln-grown samples which contained very similar quantities of thiamin had the lowest average amount of ash. Nor is the varietal effect exhibited in vitamin content reflected in the ash values. A statistical analysis of the data shows the correlation coefficient to be + .2747 and the 5% point + .497.

This absence of any significant relation between thiamin and ash is in agreement with the observations on a large number of Canadian hard red spring wheats reported by Johannson and Rich (1941). Obviously the relation found in the spring wheats is not representative for all varieties and all regions. It has been suggested that some single mineral constituent may be closely related to thiamin but that the relation between such a constituent and total ash may vary widely. Only where total ash reflects the content of this unknown mineral will a relation between thiamin and ash possibly appear.

The iron content of the winter wheats listed in Tables IV and V has been determined. Table VI gives the values for this element.

TABLE VI

THE IRON CONTENT OF WINTER WHEATS
(Iron in micrograms per gram—13.5% moisture basis)

| Variety | Location grown (Nebraska and Kansas) | | | | Average |
	Lincoln	Dodge City	Wichita	Hutchinson	
Chiefkan	49	45	44	40	44.5
Blackhull	44	42	45	37	42.0
Turkey	47	43	57	38	46.2
Tenmarq	43	40	41	34	39.5
Average	45.8	42.5	46.8	37.2	43.1

The average iron values for the Hutchinson-grown samples was lowest in the series, a rating also observed for thiamin. However, the Wichita samples which were very similar to those from Hutchinson in thiamin content exhibited the highest average for iron. Similarly, there is no apparent relation between vitamin B_1 and iron in the averages for the different varieties. The correlation coefficient for the sixteen pairs is — .020, an even lower value than that found for ash. It would appear that iron is not the unknown mineral, if such exists, which is related to vitamin B_1 content.

Table VII presents the thiamin values obtained on a number of different winter wheats. These are miscellaneous varieties grown in vari-

ous parts of the winter wheat area.   As in the other winter wheat samples Turkey and Tenmarq are appreciably higher in thiamin than Chiefkan and Blackhull.   Also, the average of all 19 samples and the range between the low and high values are very similar to those of the previous series.   These samples were not grown at the same locations and because of the effect of environment it can be anticipated that the relative values might be different if collections were made from other regions.   Extensive studies over several crop years will be required to establish varietal superiorities from the standpoint of thiamin content.

TABLE VII

THIAMIN CONTENT OF MISCELLANEOUS WINTER WHEATS
(Thiamin in milligrams per pound—13.5% moisture basis)

Variety	Source	Thiamin
Chiefkan	Falun, Kansas	1.78
Chiefkan	Guthrie, Okla.	1.67
Karmont	Billings, Mont.	1.73
Iobred	Ames, Iowa	2.00
Kanred	Bird City, Kans.	2.41
Blackhull .	Claude, Texas	1.65
Superhard Blackhull	Eagle City, Okla.	1.99
Superhard Blackhull	Meade, Kans.	1.85
Turkey	Manhattan, Kans.	2.27
Turkey	Manhattan, Kans.	2.18
Turkey	Meno, Okla.	2.56
Nebred	Manhattan, Kans.	2.08
Cheyenne	Manhattan, Kans.	2.27
Tenmarq	Preston, Kans.	2.08
Tenmarq	Manhattan, Kans.	2.70
Tenmarq	Orienta, Okla.	2.47
Kawvale	Manhattan, Kans.	1.98
Kawvale	Lincoln, Nebr.	2.71
Kawvale	Talala, Okla.	2.03
Average		2.12

Soft Wheats

Soft wheats have also been included in our survey and the results are shown in Table VIII.   These samples were all grown at the same location in Ohio.   It is interesting to note the constancy of the vitamin values for the different varieties.   Twelve of the 15 samples were within 3% of the average and may be considered to be identical.   Two of the remaining three were only about 10% below the average, leaving the last variety, Canawa, as somewhat higher in thiamin content.   Further studies would be necessary to determine whether this is a true varietal effect since only single samples are represented.

TABLE VIII

VITAMIN B$_1$ CONTENT OF SOFT WHEATS (OHIO)
(Vitamin B$_1$ in milligrams per pound—13.5% moisture basis)

Variety	Vitamin B$_1$
Trumbull	2.03
Purdue	1.99
Dawson	2.03
Fulcaster	2.10
Bald Rock	1.79
Wabash	1.87
Red Rock	2.02
Fulhio	2.09
Poole	2.02
Fultz	2.05
Gladden	1.97
Thorne	2.05
American Banner	2.01
Yorkwin	2.05
Canawa	2.38
Average	2.03

West Coast Wheats

Table IX shows the values obtained on a number of wheats grown on the west coast. They represent several different types and varieties and have an average thiamin content similar to that of the winter wheats grown in the midwestern states. The range of values is also very similar.

TABLE IX

VITAMIN B$_1$ CONTENT OF WEST COAST WHEATS
(Vitamin B$_1$ in milligrams per pound—13.5% moisture basis)

Variety	Vitamin B$_1$
Baart	2.31
Baart No. 17	2.24
Baart No. 38	2.11
Federation	1.76
Kharkof	1.94
Brevon	1.77
Turkey	1.85
Blue Stem	2.44
Ridit	1.97
Rex	2.28
Hymar	2.36
Oro	2.04
Average	2.09

Canadian Wheats

Table X gives the thiamin values found in several varieties of Canadian wheats. Each variety was grown at Winnipeg and at one

other Canadian point. In all cases the Winnipeg samples were higher in vitamin content than the corresponding varieties grown elsewhere. This is another illustration of the profound effect of environment. Two of the varieties, Thatcher and Renown, were common to the Minnesota samples shown in the first table. The values for the Winnipeg samples were similar to those grown in St. Paul, while those grown at the other Canadian stations were almost identical with the Crookston samples.

TABLE X

VITAMIN B_1 CONTENT OF CANADIAN WHEATS

(Vitamin B_1 in milligrams per pound—13.5% moisture basis)

Variety	Source	Vitamin B_1	Ash
			%
Red Bobs	Winnipeg	2.70	1.44
Red Bobs	Airdrie, Alta.	1.84	1.11
Thatcher	Winnipeg	2.64	1.81
Thatcher	Saltcoats, Sask.	2.10	1.45
Apex	Winnipeg	2.38	1.66
Apex	Kelvington, Sask.	1.99	1.45
Garnet	Winnipeg	2.28	1.48
Garnet	Lacombe, Alta.	1.45	1.62
Reward	Winnipeg	2.61	1.53
Reward	Coderre, Sask.	2.35	1.50
Renown	Winnipeg	2.81	1.79
Renown	Gilbert Plains, Man.	2.24	1.33
Mindum	Winnipeg	2.69	1.47
Average of Winnipeg samples		2.59	1.60
Average of other samples		1.99	1.41
Average of total samples		2.31	1.51

The samples include one variety of durum wheat, Mindum, the thiamin content of which was about the same as the average for the spring wheats. A similar observation was made on two durum samples grown in St. Paul. These were similar to spring wheats from the same location.

The sample of Garnet wheat grown at Lacombe contained the lowest amount of thiamin which we have observed. This is nearly twice as high, however, as the 0.5–0.6 International Unit reported by Booth (1940) for samples of Manitoba No. 3 and No. 1 Dark Northern Spring.

The ash content of these Canadian wheats has also been determined and the values are included in the table. There was no significant correlation between thiamin and ash, the correlation coefficient being + .402 and the 5% point, + .553. It was interesting to note that, with the exception of the Garnet variety, the Winnipeg-grown samples were higher in both thiamin and ash than the corresponding varieties grown at the other locations. Despite this there was no significant relation between the vitamin and ash in either group.

Other Cereal Grains

Table XI summarizes the averages for the various wheats examined and compares these values with those of other cereal grains. With the exception of oats, these other grains were similar to wheat in thiamin content. While at least six varieties of each were studied the number of locations where they were grown was too limited to justify the acceptance of their values as representative averages. The oats, barleys, and many of the corns were obtained from stations which showed a high vitamin content in wheats. For this reason nationwide averages might be expected to be somewhat lower.

TABLE XI

Thiamin Content of Cereal Grains
(Thiamin in milligrams per pound)

Grain	Number of samples	Vitamin B_1 Average	Vitamin B_1 Range
Spring wheats			
Minnesota	24	2.75	2.01–3.49
Canada	13	2.31	1.45–2.81
Winter wheats	35	2.17	1.65–2.71
West Coast wheats	12	2.09	1.76–2.44
Soft wheats	15	2.03	1.79–2.38
Corn			
Yellow	6	2.44	2.19–2.82
White	6	2.76	2.24–3.04
Oats	6	4.20	3.68–4.90
Rye	6	2.11	1.88–2.28
Barley	6	2.95	2.58–3.33
Grain sorghums	7	2.68	1.93–3.97

Summary

The evidence presented indicates that the thiamin content of wheat is influenced by the wheat type, variety, and environment. In general, durum and spring wheats contained the largest amount of vitamin B_1, followed by hard winter and soft wheats in this order. A significant effect of variety was observed in a series of winter wheats. Environment is an important factor, since the same varieties grown at different locations differed widely in content of thiamin.

In a series of spring wheats a high correlation between thiamin and ash was observed. This relation did not prevail in the instance of other spring wheats or a series of winter wheat varieties. Corn, rye, barley, and sorghum were found similar to wheat in their thiamin contents. Oats contained somewhat more of this vitamin.

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THIAMIN IN THE PRODUCTS OF WHEAT MILLING AND IN BREAD [1]

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(Read at the Annual Meeting, May 1941)

Of the many vitamins known to be necessary for man, wheat contains several members of the water-soluble vitamin B complex, thiamin, riboflavin, nicotinic acid, vitamin B_6, and pantothenic acid. It also contains the fat-soluble vitamin E. Thiamin (vitamin B_1) is very important from the viewpoint of flour millers since wheat is regarded as an excellent source of this vitamin.

If the estimate of 6½ ounces is accepted as the average daily per capita consumption of flour, and if it is assumed for illustration that it is all whole-wheat flour, the thiamin contribution to the daily diet will be about 0.9 mg. This is one-half the daily requirements of a moderately active man according to figures recommended by the Committee on Food and Nutrition of the National Research Council (1941).

Wheat flour has not been contributing this amount of thiamin to the human dietary in the past, however, as only a small portion of the flour consumed in this country is actually made from the entire grain. There-

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[1] No. 31, Journal Series, General Mills, Inc., Research Laboratories.

fore, any practical evaluation of wheat for its nutritional service to man must be based upon commercial types of white flour rather than upon whole-wheat flour.

The Distribution of Thiamin in Products of Milling

As a whole wheat is recognized as an excellent source of thiamin, it is important to examine the wheat kernel and the products of milling to determine the distribution of this vitamin. The distribution is of special interest in view of the current program of enriched flour production.

The vitamins and minerals of wheat are not uniformly distributed throughout the kernel, but are highly concentrated in certain tissues, while others contain only small amounts. The outer layers and the germ are high in these factors, the endosperm relatively low. Patent flour, comprised almost entirely of endosperm, has about one-seventh of the thiamin concentration of whole-wheat flour. Of the total thiamin in the wheat kernel, the endosperm contains about 24%, the embryo about 15%, and the outer layers of the kernel about 61%.

The values just cited are not based on milling yields but on calculations which show the thiamin distribution between endosperm, embryo, and bran. They do not give a true picture of the thiamin content of the products of milling, since it is impossible to make a sharp separation between the different structures in the wheat kernel in the commercial process of milling.

Analyses of products of commercial wheat milling have been made to determine the distribution of thiamin in certain well-known commercial products. The thiamin content of the products from three different hard-wheat mixes is shown in Table I. These values are in good agreement with those reported by Schultz, Atkin, and Frey (1939), considering the variability in wheat and in milling practices.

TABLE I

THIAMIN (VITAMIN B₁) CONTENT OF PRODUCTS OF COMMERCIAL WHEAT MILLING
(Samples taken from three different hard wheat mixes)

Sample	Thiamin (mg per pound)		
	Mix 1	Mix 2	Mix 3
Cleaned wheat	2.18	2.35	2.31
Patent flour	0.29	0.29	0.35
First clear flour	1.28	1.31	1.50
Second clear flour	6.22	6.05	4.57
Red dog flour	13.60	14.42	12.32
Germ	10.65	10.20	—
Shorts	4.31	9.07	10.28
Bran	4.04	3.73	4.98

The wheat mixes were similar in thiamin content, two of them being practically identical. The patent flours are also similar as are the first clear and red dog. There is greater variation in the other products of milling from different wheat mixes, especially the shorts. Variations in milling practice can account for large differences in thiamin content of these materials. By taking average values for the milling yields, the percent of the total wheat thiamin in each of these mill products has been calculated. These values are shown in Table II.

TABLE II

Thiamin (Vitamin B₁) Distribution in Products of Commercial
Wheat Milling
(Averages of samples from several millings)

Sample	Milling yield Percent of cleaned wheat (approximate)	Thiamin content Mg per pound	Calculated as percent of total thiamin in wheat
Patent flour	63.0	0.31	8.0
First clear flour	7.0	1.36	3.9
Second clear flour	4.5	5.61	10.0 ·
Red dog flour	4.0	13.45	22.0
Germ	0.2	10.40	0.9
Shorts	12.3	7.89	39.6
Bran	9.0	4.25	15.6
Cleaned wheat	100.0	2.28	100.0

Patent flour, representing 63% of the cleaned wheat, contains only 8% of the total thiamin of the whole grain, whereas second clear flour, representing only 4.5% of the wheat, contains 10% of the thiamin. Red dog flour contains 22% of the thiamin.

The percent of thiamin represented by germ appears to contradict the previous statement respecting the amount of thiamin in the embryo. This apparent discrepancy is due to the fact that commercial milling does not permit the recovery of all the germ contained in the wheat. In the case of the samples shown in· Table II, less than 10% of the total germ was recovered in the germ stream. The other 90% is in the feeds.

The figures presented in Table I were derived from the products of two mills. Since milling practices differ in different mills the data cannot be regarded as representative of commercial milling operations. Accordingly, our studies were extended to include a wider variety of products. The results shown in Table III were obtained on samples from nine different mills. Ash values are also shown to indicate the approximate degree of extraction.

TABLE III

THIAMIN AND ASH IN PRODUCTS OF COMMERCIAL WHEAT MILLING
(Includes products from nine mills)

Sample	Thiamin (mg per lb) [1]		Ash (%)	
	Average	Range	Average	Range
Cleaned wheat	2.20	1.97– 2.43	1.56	1.40–1.75
Patent flour	0.32	0.23– 0.48	0.40	0.36–0.43
First clear	1.20	0.50– 1.66	0.68	0.44–0.85
Second clear	3.76	2.00– 5.34	1.33	0.88–2.34
Red dog	11.74	10.39–13.45	2.83	2.44–3.70
Shorts	8.03	5.60– 9.83	4.35	3.63–5.16
Bran	3.99	2.76– 4.98	6.52	6.30–6.94

[1] All samples contained about 11% moisture at time of analysis.

In general the average values for thiamin are in good agreement with those obtained from the products of a single mill (Table I). There is, however, a rather wide range in the vitamin content of each of the different grades of flours and feeds. This cannot be attributed to the amount of thiamin present in the individual wheats since all are within 10% of the average.

Since ash values reflect to a considerable extent the types of milling, it is of interest to compare these values with those for thiamin. Such a comparison is presented in Figure 1.

There is a close parallelism between thiamin and ash in the patent,
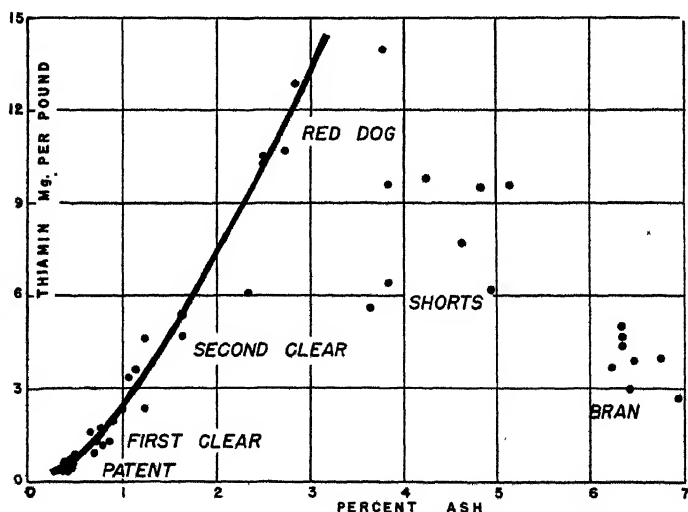
Fig. 1. Relation of thiamin to ash in products of wheat milling.

clear, and the red dog flours.   In the shorts and bran, however, these relations disappear and the further increase in ash shows a marked and irregular decrease in thiamin.   This can be in part explained by the relatively low thiamin content of bran, a considerable amount of which is present in the shorts.

To show the relationship between long extraction and thiamin content, the values given in Table II have been calculated differently in Table IV.

TABLE IV

RELATION OF PERCENT OF EXTRACTION TO PERCENT OF TOTAL THIAMIN IN CLEANED WHEAT

(Theoretical values obtained by calculation, not by actual production of long extraction flour)

Sample	Extraction— percent of cleaned wheat	Percent of total thiamin in cleaned wheat
1. Patent flour	63.0	7.8
2. Patent flour plus first clear	70.0	11.8
3. (2) plus second clear	74.5	23.8
4. (3) plus red dog	78.5	48.8
5. (4) plus germ and shorts	91.0	85.4
6. (5) plus bran (entire wheat)	100.0	100.0

It will be noted that an addition of 7% of first clear to patent flour raises the thiamin content of the resultant straight-grade flour to 50% above the patent.   A further addition of 4.5% of second clear doubles the thiamin content of the straight grade.   Another addition of 4%, in the form of red dog, again doubles the thiamin content.   It must be pointed out that the thiamin percentages shown in this table for the long-extraction products were obtained by calculation and not by actual milling to such high extractions.   The same values are shown to better advantage in a curve (Fig. 2) where percent of total wheat is shown on the horizontal axis and percent of total thiamin in wheat on the vertical axis.   Below 70% extraction the curve rises gradually, while a sharp rise occurs between 73% and 79%.   Above 80 the slope again changes, and as the curve approaches 100 each increment of increase in extraction brings less increase in thiamin.   This is due to the relatively low thiamin content of the outer bran layer.

Our values and those reported by others show clearly that the more refined flours are lower in thiamin.   Since ash is a criterion of refinement, it seemed desirable to determine the correlation between the two factors.   Hoffman, Schweitzer, and Dalby (1940) have previously noted a relationship between ash and thiamin content.

In the course of our investigations to determine the distribution of thiamin in the products of wheat milling a large number of mill streams
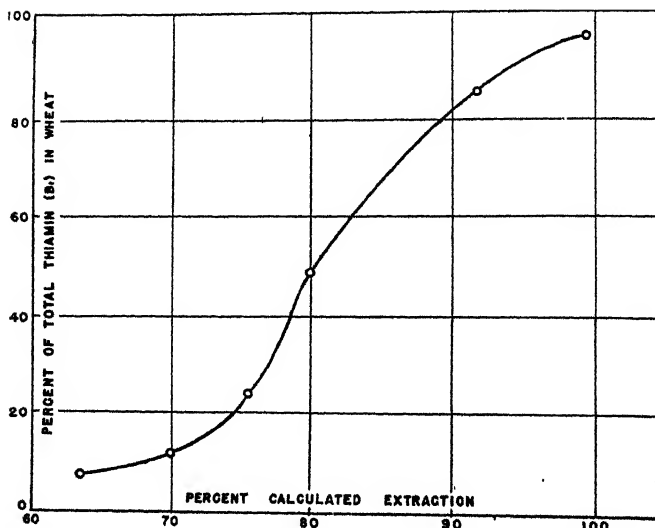
Fig. 2. Relation of thiamin to percent extraction of wheat.

have been examined in addition to commercial products of milling. These mill streams have been obtained from mills all over the United States and, accordingly, represent a variety of milling operations performed on many types of domestic bread wheats. The ash contents of the streams range from the low values of patent flour to the high values of low-grade flours and feeds. A high positive correlation has been found between thiamin and ash contents. Figure 3 shows the relationship graphically.

For the entire population the coefficient is $r = + .9498$ indicating a very high correlation. When three bran duster flours high in ash and relatively low in thiamin are omitted, the correlation becomes $r = + .9716$ for the curves shown in Figure 3. The solid line represents all samples, the dotted line all except the three bran duster streams. Statistically there is a significant difference between these two correlations and hence in the calculation of r there is justification for the omission of the three samples noted above, since it is clear that they belong in a separate classification. Failure of the three bran duster samples to fall in line with the other mill streams and the fact that the curve flattens in the high ash range show clearly that in practical mill operations ash content cannot be used as the sole criterion for selecting high vitamin B_1 mill streams unless it is known that the streams are not rich in bran tissue.

The high correlation between ash and thiamin prevails until the ash content reaches about 2.5%. With higher ash the thiamin increase is
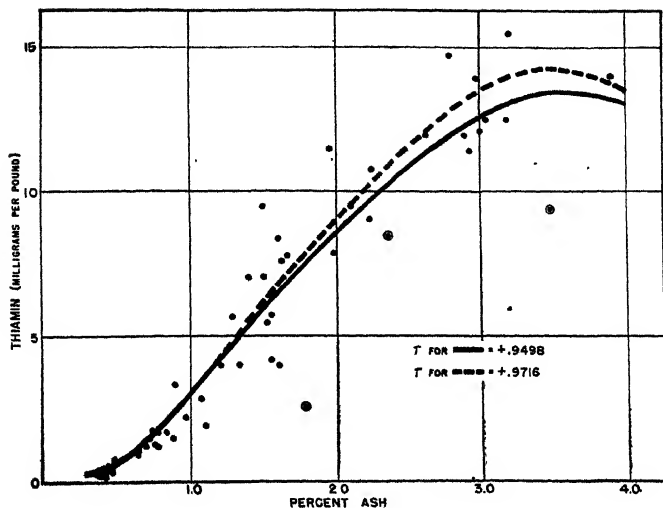
Fig. 3. Relation of thiamin to ash in mill streams.

somewhat less.   Reference to Table II shows that bran contains less thiamin than second clear, red dog, germ, and shorts.   This is due to the relatively low thiamin content of the outer bran or pericarp.   Since bran is very high in ash it is to be expected that streams rich in bran tissue will not show the same relation between thiamin and ash as do the other streams.

The data show clearly that much of the thiamin in whole wheat is contained in the fractions of milling which normally go into the animal feeds.   Low-grade or red dog flour is especially high in thiamin content, exceeding germ in the samples tested in this study.   This was especially interesting, as germ has been frequently reported as an unusually rich source of thiamin.

Microscopic examination of the low-grade flours separated in the laboratory did not reveal the predominance of any specific tissue of the wheat kernel.   Aleurone and branny layers and starch cells were identified.   The outer bran layer (pericarp) could not have been present in significant amount, since actual tests of purified bran have shown that it is not rich in thiamin.

These observations have led us to postulate that the distribution of thiamin in wheat may be due largely to the conditions which prevail during the period of growth when the kernel is maturing.   During the milk stage the kernel has a high water content, and since thiamin is water-soluble, it is conceivable that the distribution *may* be more uniform at that time, although evidence is not now available to support this

speculation. As the kernel ripens, water moves from the inside to the surface and is lost. It is believed that this movement of water may result in translocation of the vitamin to the layers of the kernel surrounding the endosperm, where it becomes concentrated as the water evaporates. Thiamin does not become concentrated, however, in the outer bran layer, or pericarp, and tests of pure bran do not show high thiamin. This suggested explanation respecting disposition of thiamin might account for failure to find high vitamin concentration in the outer tissue. Concentration of thiamin in the layers of tissue just underneath the pericarp would also probably account for the rapid increase in thiamin when milling extraction is increased from 73% to 79%.

Thiamin Content of Whole Wheat Bread

In addition to the examination of several mill products, whole-wheat flours have been tested for thiamin. Thirty-one samples of commercial whole-wheat flours collected from bread-wheat mills distributed about the United States were found to contain an average of 2.27 mg of thiamin per pound. The values ranged from 1.92 to 2.53, a much narrower range than found by Nordgren and Andrews (1941) in samples of whole wheat obtained from experimental plots. The latter ranged from 1.4 to 3.2. The blending of large lots of commercial wheats in making wheat mixes for grinding into whole-wheat flour is undoubtedly the explanation of the smaller variations in thiamin in commercial whole wheat flours.

Each of the 31 samples of commercial whole-wheat flours was baked in the laboratory with a commercial formula. The whole-wheat bread averaged 1.36 mg of thiamin per pound of fresh bread. The relationship between thiamin in flour and thiamin in fresh bread was quite uniform in comparisons of individual samples of whole-wheat flour and bread. The concentration of thiamin in the fresh bread containing 38% moisture averaged 60% of that in the flour, the individual samples ranging from 58% to 61%. This does not, of course, mean that 40% of the thiamin is lost during baking, since the lower concentration in the bread is due in a large degree to the dilution of the vitamin by the water in the bread. Actual vitamin losses were not calculated since the thiamin content of the ingredients other than flour was not determined. The study was made in order to evaluate approximately the thiamin content of commercial whole-wheat loaves.

During the course of our investigations two samples of foreign " war flours " were made available. A sample from Switzerland contained 0.87 mg per pound, and one from Italy contained 1.14 mg per pound.

Summary

The distribution of thiamin in wheat as determined by the analysis of the products of commercial milling is reported and discussed. The major part of the vitamin occurs in those tissues of the wheat kernel just beneath the outer bran. These tissues are responsible for the high thiamin content of the feeds such as red dog and shorts. A possible explanation for this distribution is presented.

There is a high correlation between thiamin and ash in the milling fractions having 2.5% or less of ash. Above this value the correlation is less significant because of the varying amounts of outer bran tissues, which are low in thiamin.

The thiamin contents of whole-wheat flours and breads prepared therefrom are reported.

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THE IRON CONTENT OF CEREALS [1]

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(Read at the Annual Meeting, May 1941)

The iron content of whole cereal grains, especially wheat, has long been recognized as a property of considerable nutritional value. Medical authorities have advocated consumption of whole-wheat products as a means of increasing the amount of iron supplied by the average human dietary.

Since much of the iron in wheat is distributed throughout the tissues adjacent to and constituting the bran layer, the milling of flour yields a product that is relatively low in this nutritive element. Cognizance of this has been taken by those agencies interested in establishing

standards for "enriched" flour and bread, with the result that the addition of an assimilable iron compound has been made a prerequisite for these recently proposed products.

This action has introduced a new problem for many of the laboratories serving the flour manufacturer and allied trades. In the past the absence of any great demand for analyses of iron in cereal products has confined such activities to a few laboratories interested in problems of a research nature. From this status the demand has suddenly increased so that today methods of analysis are of interest to many other laboratories, notably those engaged in the control of manufactured cereal products.

Discussion of Analytical Methods for Iron

An examination of the literature reveals a rather confusing mass of information on the subject of the iron analysis of foods. Numerous observations are reported about the difficulties which attend the use of various methods and many modifications have been proposed for the elimination of factors that adversely affect the successful operation of these methods. In several instances the modifications have so greatly complicated the procedure that the analyst must be painstaking and expert to avoid the introduction of serious errors. With this in mind the writers have examined some of the problems involved, especially as they pertain to cereals and cereal products.

The widespread use of colorimetric methods is tacit acknowledgment of the inadequacies of the usual volumetric and gravimetric methods. In most instances the amount of iron contained in cereals is too low to permit convenient application of the latter procedures. Thus, one finds an almost universal employment of colorimetric techniques, which are better adapted to the analysis of relatively minute quantities. Many substances that produce colored compounds with iron have been used. Among these are ferricyanide and sulfocyanate in the inorganic, and o-phenanthroline, mercaptoacetic acid, and α,α'-dipyridyl in the organic fields. Of these substances, the sulfocyanate has been most extensively studied and widely used, and only recently has α,α'-dipyridyl been employed outside its special application to the determination of "available iron" in foods.

The use of either of these reagents requires that the iron compound under examination be present in a reactive form. With cereals it must be contained in a solution free from substances which inhibit the development of color when treated with the reagent. Prominent among the inhibitors is pyrophosphate, which forms during the dry ashing of the sample. In the presence of pyrophosphate iron does not react completely with sulfocyanate and a quantitative evaluation of the iron is prevented.

This inhibitory effect can be eliminated by employing so-called "wet ashing" procedures, which prevent the formation of pyrophosphate, or by hydrolysis of the solution of ash prior to the addition of the reagent. Hydrolysis can be effected by heating either with acids or alkali. Elvehjem (1930) proposed the use of alkali for samples having a high pyrophosphate content while Farrar (1935) and Shackleton and McCance (1936) employed hydrochloric acid. Stugart (1931) expressed a preference for the acid method and pointed out that the use of alkali introduced variable quantities of foreign iron compounds. Hoffman, Schweitzer, and Dalby (1940) used electrolytic caustic to avoid iron contamination. In a recent study of the α,α'-dipyridyl method Jackson (1938) eliminated pyrophosphate interference with hydrochloric acid but apparently found that hydrolysis was slow since the ash solution was heated at 80°C for 18 hours.

A standard method for the hydrolysis of pyrophosphates in analytical practice is fusion with sodium carbonate. Since this compound can be readily purified by crystallization from the bicarbonate, this method offers a means of removing pyrophosphate from cereal ash. The writers have studied this possibility by mixing the ash with the carbonate and fusing the mixture in a platinum crucible. Two minutes' fusion was adequate to destroy any pyrophosphate as evidenced by instant and complete development of color with dipyridyl. No serious contamination with iron was found when the same procedure was applied without the ash.

From the standpoint of convenience, however, the fusion with carbonate is not the most desirable since the fusion vessels must be kept scrupulously clean and a quantitative transfer of the ash from the ashing dish is required. Also, the ash should be thoroughly mixed with the carbonate, preferably by grinding, and this step requires considerable care. With the proper technique, however, excellent results can be obtained.

The use of acid hydrolysis offers by far the simplest method. Solution of the ash can be effected directly from the ashing crucible and a few minutes' heating is all that is required to destroy interfering salts.

For developing the color of the iron α,α'-dipyridyl has been chosen since it is convenient to use and the color is stable and easily measured. The iron solution is first adjusted to the proper pH by the addition of a buffer solution and any ferric iron is then reduced by adding a solution of hydroquinone. The dipyridyl solution is then added and the color measured. In order to compensate for any traces of iron which may be present in the various reagents a blank is prepared and this solution is used for adjusting the photoelectric colorimeter.

In addition to the interference of pyrophosphates a second factor reputed to cause analytical errors has been given considerable attention in the literature. This concerns the loss of iron by volatilization during the ashing process. Jackson (1938) reported that iron losses can be avoided only by "wet ashing" with a mixture of nitric, sulfuric, and perchloric acids. Hoffman, Schweitzer, and Dalby (1940) moistened samples of bread with alkali prior to ashing and thereby prevented losses which otherwise were very large. Farrar (1935) ashed samples with calcium carbonate to avoid contamination by the acids used in "wet ashing." He found, however, that losses of iron during the usual dry ashing were not always evident since the analysis of a sample of dog bread gave the same result when carbonate was used and when it was omitted.

For the cereal chemist it is of considerable advantage to be able to analyze the ash obtained from the regular ash determination. Accordingly, the writers have investigated the loss of iron as evidenced by the recovery of added iron compounds. These have included ferric chloride since losses are generally attributed to the formation of this somewhat volatile iron salt. The results are shown in Table I.

TABLE I

THE RECOVERY OF ADDED IRON IN THE ANALYSIS OF FLOUR

Sample	Added iron	Calculated	Found
	%	%	%
Flour	none	—	0.0009
Flour plus iron pyrophosphate	0.0060	0.0069	0.0071
Flour plus iron phytate	0.0035	0.0044	0.0042
Flour plus reduced iron	0.0035	0.0044	0.0042
Flour plus iron mucate	0.0035	0.0044	0.0044
Flour plus ferric chloride	0.0035	0.0044	0.0045

These analyses were made by ashing overnight the samples at about 575°, heating the ash with a few milliliters of dilute hydrochloric acid, making to volume, and measuring the color developed by dipyridyl in an aliquot of the solution. There is no evidence of any loss of iron since the differences between the found and calculated values are within the experimental error of the method.

Similar excellent recoveries have been obtained from the analyses of breads made from flour containing added iron. These experiments included one series of breads made with varying amounts of salt, an ingredient which might promote a loss of iron during ashing. Table II presents the results.

There seems to be no evidence that normal amounts of salt cause losses of iron under the analytical conditions employed.

TABLE II

EFFECT OF SALT ON THE IRON ANALYSIS OF BREAD

Salt in bread	Iron
%	%
0	0.0122
1	0.0122
2	0.0119
4	0.0121

Iron Content of Wheat and Its Products of Milling

Using the dry ashing procedure and the dipyridyl method, samples of various wheats and mill products have been examined. Wheats normally contain between 30 and 50 μg of iron per gram although values as low as 27 and as high as 60 have been obtained on a few samples. Most bread flours of patent grade range between 6 and 9 μg per gram or 2.7 to 4.1 mg per pound. Occasionally iron values as low as 4 μg per gram and as high as 12 have been observed but these appear to be unusual.

The other products of milling vary widely in their content of iron. Table III shows the results found by the examination of all the products obtained from a single commercial milling of wheat.

TABLE III

THE IRON CONTENT OF THE PRODUCTS OF WHEAT MILLING

(Basis 13.5% moisture)

Product	Ash	Iron	Iron in ash
	%	%	%
Patent flour	0.41	0.00078.	0.190
First clear	0.82	0.0019	0.232
Second clear	2.34	0.0062	0.265
Red dog	3.70	0.0113	0.306
Shorts	4.18	0.0127	0.305
Bran	6.53	0.0126	0.193
Germ	4.14	0.0086	0.208
Wheat	1.65	0.0037	0.224

While in most instances there is an increase in the iron content as the ash increases, this relation does not hold for all the mill products. Thus, bran with nearly 50% more ash than shorts actually contains about the same amount of iron. Similarly, germ is considerably lower in iron than shorts although its ash content is approximately the same. This suggests that the mechanism responsible for the distribution of iron in the wheat berry may differ with respect to the other mineral constituents. It demonstrates that the ash content does not serve as an accurate index of the quantity of iron present. This was also

observed in the case of patent flours where widely varying amounts of iron were found in samples of similar ash content. The last column in the table shows how the ratio of iron to ash varied in the particular products examined. The ratio is lowest in the patent flour and bran and highest in red dog and shorts. Wheat occupies an intermediate position, as of course it should, to account for the ratios in its mill products. On the basis of approximate mill yields an average ratio can be calculated for the different fractions in the proportion of their amounts in wheat. This calculated value is 0.222, which agrees well with the observed value of 0.224.

These findings are not entirely in accord with the results reported by Sullivan and Near (1927), who analyzed a series of mill products and found a rather constant relationship between ash and iron. In the samples which they analyzed the iron content of the ash was about 0.15% with less than 10% variation from this average in the different mill fractions.

In order to determine whether our results were due to some unusual distribution of iron in the particular wheat examined or to differences in milling operations, the present studies were extended to include a number of mills operating on different wheat mixes. Table IV presents the averages obtained on the fractions from ten different mills.

TABLE IV

THE IRON CONTENT OF FRACTIONS OF WHEAT

(Dry basis)

Mill fraction	Average iron content	Iron in ash	
		Average	Range
	ug/g	%	%
Patent flour	8.4	0.180	0.125–0.239
First clear	17.4	0.219	0.165–0.250
Second clear	38.7	0.258	0.214–0.290
Red dog	96.2	0.302	0.284–0.337
Shorts	139.0	0.281	0.249–0.335
Bran	146.2	0.206	0.187–0.228
Germ	91.3	0.192	0.174–0.210
Wheat	41.6	0.232	0.198–0.262

While the actual values are not the same as those given in Table III the relative differences are similar. In each case the average percentages of iron in the ash are lowest for the patent flour and bran and highest for the low-grade and finely ground feeds. Germ is also low and wheat again occupies an intermediate position. The last column shows the range of values found. It will be noted that patent flours vary widely, since for a 0.40 ash grade the range of 0.125 to 0.239 means an iron variation of from 5 to 9.5 μg per gram.

In individual fractions, that is, those obtained from a single wheat mix, these average ratios of iron to ash will not always prevail. There is a considerable difference between different wheats and mills. In two of the series presented above the ratio was much more constant than the averages shown. One of these was a series kindly supplied by Dr. Betty Sullivan. Iron analyses were in excellent agreement between the two laboratories and the ratios of iron to ash in the patent and clears were the same as that of the wheat. Bran, and especially germ, ran somewhat lower and shorts tended to be higher, but differences were much less marked than the averages shown in the table.

Experimental

The analytical method employing carbonate fusion of the ash has already been described by the American Association of Cereal Chemists (1941). The method using acid hydrolysis was as follows:

A 2- to 5-gram sample was ashed in a porcelain crucible placed in a muffle furnace. The maximum temperature of the furnace was 575°C and total ashing time was 16 to 18 hours. After cooling, 2 ml of concentrated hydrochloric acid (cp quality) was added and the crucible, covered with a watch glass, was gently heated for a few minutes until the ash was dissipated. (Ash from whole wheat will contain carbon particles which are removed by filtration after diluting to volume. These particles contain no measurable amount of iron.) The ash solution was then transferred to a 100-ml volumetric flask and after thorough washing with distilled water was made up to volume.

Ten ml of this solution was placed in a colorimeter tube and the following solutions added: 2 ml of $2\frac{1}{2}\%$ hydroquinone, 5 ml of acetate buffer, and 2 ml of 0.1% α,α'-dipyridyl. After thorough mixing, the color of the solution was read in an Evelyn photoelectric colorimeter.

Summary

A simple method using α,α'-dipyridyl for the determination of iron in cereals and cereal products is described. Pyrophosphate interference is eliminated from the ash either by fusion with sodium carbonate or by heating with hydrochloric acid. Losses of iron during dry ashing have not been observed. The iron analyses of wheat and its products of milling are reported.

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BOOK REVIEW

Chemistry of Food and Nutrition, Sixth Edition. By Henry C. Sherman. Published by the Macmillan Company, New York, N. Y. 1941. 660 pages. $3.25.

This new edition of a standard book retains many of its well known characteristics and contents but has a changed and more attractive format. Since much new information in the field of nutrition has been accumulated in the four years following the last revision, the author has had to choose topics for deletion from both old and new data in order to retain the size of the former edition. There may not be universal approval of the choices made, particularly in the treatment of the intermediary metabolism and in the elimination of most of the purely chemical discussion. Doubtless many readers of the book will applaud this trend toward the practical or applied type of treatise, even though the supply of books of the latter type is not inadequate, and there are very few indeed of the more scientific variety.

The division of space allotted to the various topics is as follows: review of the chemistry and nutritive function of carbohydrates, fats, and proteins, 12%; digestion and metabolism, 7%; energy requirement, 12%; protein requirement, 4%; minerals, 17%; vitamins, 21%; reproduction and growth, 5%; interpretation in terms of human feeding, 12%; and tables of food composition, 3%.

A generous list of references is printed at the end of each of the chapters. For most readers, however, more value might be derived from these references if they were presented as documentation of the statements in the text.

The author has adopted a philosophic and at times forensic style, particularly marked in the discussion of the calcium requirement, in presenting his interpretation of the facts of nutrition—an unusual privilege which might well be conceded in this case. The choice of data cited may not be universally accepted as the best by other workers in the field, but since choice had to be made, the author naturally selected that which was in his judgment most significant.

Certain interesting discrepancies grew out of this condition. For instance the discussion of the need for thiamin contains guarded skepticism as to the advantage of increased intake beyond the actual requirement and the general conclusion that a reasonably intelligent choice of diet will provide adequate thiamin. But much emphasis is given to the desirability of riboflavin intakes far in excess of the minimum, based chiefly upon the author's experiments with rats fed diets containing various amounts of milk.

A curious omission occurs in that there is no mention of the well known toxic effects of excess intake of vitamin D. Similarly, although the work of Jeans and Stearns upon the vitamin D requirement of infants is frequently cited, there is no reference to their experience with adverse effects of administration to infants of doses greater than 1800 U. S. units daily. This failure to include a mild warning of the danger of hypervitaminosis D is unfortunate in a book which may be for many students the sole source of knowledge of the newer nutrition.

The tables in the text as well as in the appendix are concise and complete. The table of mineral composition of foods is a unique contribution for which this book has long been esteemed and the vitamin table is an excellent compilation, expressed in terms of weight units for the most part. Another good feature of the book, important in so authoritative a volume, is the use of the proper chemical names for vitamins of known structure.

No student of nutrition should miss the pleasure and profit of reading this new edition of so important a pioneer book.

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